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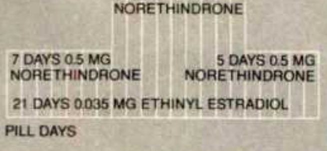
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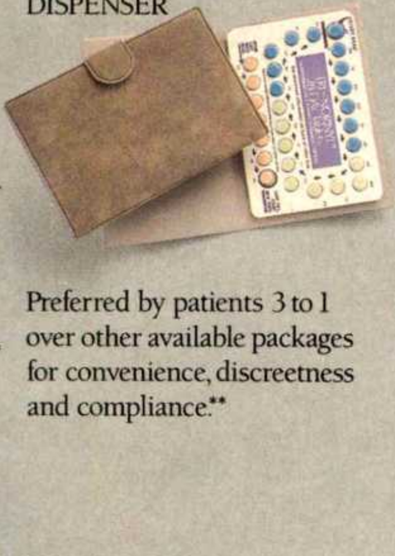

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THE NATURE OF TRI-NORINYL®

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[*] 1/35 formulations contain 1.0 mg norethindrone with 0.035 mg ethinyl estradiol. Data available from Syntex Laboratories, Inc.

[**] Based on a nationwide, independently conducted survey of prospective oral contraceptive patients. Data available from Syntex Laboratories, Inc.

Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. Please see brief summary of full prescribing information on next page.

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THE NATURE OF
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TRI-NORINYL® 21-Day Tablets (7 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, 9 green tablets of norethindrone 1.0 mg with ethinyl estradiol 0.035 mg, 5 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg)

TRI-NORINYL® 28-Day Tablets (7 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, 9 green tablets of norethindrone 1.0 mg with ethinyl estradiol 0.035 mg, 5 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg; 7 inert tablets)

ORAL CONTRACEPTIVE (O.C.) AGENTS

Indications Prevention of pregnancy. *DOSE-RELATED RISK OF THROMBOEMBOLISM.* Because studies have shown a positive association between OC estrogen dose and risk of thromboembolism, it is prudent to minimize estrogen exposure. Prescribe an OC with the least amount of estrogen compatible with an acceptable pregnancy rate and patient acceptance. Start new users on OCs containing 0.05 mg or less of estrogen.

Contraindications 1. Known or suspected pregnancy (see Warning #5). 2. Thrombophlebitis or thromboembolic disorders. 3. Past history of deep vein thrombophlebitis or thromboembolic disorders. 4. Undiagnosed abnormal genital bleeding. 5. OCs should not be used by women who have or have had any of the following: a. cerebral vascular or coronary artery disease, including myocardial infarction. b. known or suspected carcinoma of the breast. c. known or suspected estrogen dependent neoplasia. d. benign or malignant liver tumor that developed during use of OCs or other estrogen containing products.

WARNINGS: Cigarette smoking increases the risk of serious cardiovascular side effects from OC use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use OCs should be strongly advised not to smoke.

The use of OCs is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitioners prescribing OCs should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with OC use is established. One study demonstrated an increased relative risk for fatal venous thromboembolism and several studies demonstrated it for non-fatal venous thromboembolism. They estimate that OC users are 4-11 times more likely than nonusers to develop these diseases without evident cause. One British study reported an excess death rate of 40% in OC users, most of which resulted from cardiovascular disease. Another British study showed a lower death rate in OC users than controls; an increase in cardiovascular deaths was seen but was not statistically significant. A U.S. prospective study failed to disclose increased mortality rates from cardiovascular disorders, but a subset analyzed as a retrospective, case-control study showed significant increases in venous thromboembolism. CEREBROVASCULAR DISORDERS. Two American studies demonstrated an increased relative risk for stroke not shown in prior British studies. In an American study of cerebrovascular disorders in women with and without predisposing causes, relative risk of hemorrhagic stroke was estimated as 2.0 times greater and thrombotic stroke as 4-9.5 times greater in users than nonusers. A British long-term follow-up study reported in 1976 a highly significant association between OC use and stroke. Another study had suggested an association in 1974, but the number of cases was too small to estimate the risk. Subarachnoid hemorrhage has been shown to be increased by OC use in British and American studies. Smoking alone increases incidence of these accidents; smoking and pill use appear to increase risk more than either alone.

MYOCARDIAL INFARCTION (MI). Increased relative risk of MI associated with OC use has been reported. One British study found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing MI, regardless of OC use. OCs were an additional risk factor. In terms of relative risk, it has been estimated that nonsmoking OC users (smoking is considered a major predisposing condition to MI) are twice as likely to have a fatal MI as nonsmoking nonusers. OC users who are smokers have a 5-fold increased risk of fatal infarction compared to nonsmoking users, and a 10-12-fold increased risk compared to nonsmoking nonusers. The number of cigarettes smoked is important. In determining the importance of these relative risks, baseline rates for various age groups must be considered. (Estimates are based on British vital statistics which show acute MI death rates 2-3 times less than in the U.S. so U.S. death rates could be higher.) Importance of other predisposing conditions in determining relative and absolute risks has not been quantified; other synergistic actions may exist. RISK OF DOSE. Using data from several national adverse reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to estrogen dose in OCs. OCs with 0.1 mg or more of estrogen were associated with a higher risk of thromboembolism than those containing 0.05-0.08 mg but quantity of estrogen may not be the sole factor. This was supported by a U.S. study. A British study found a positive association between dose of progestogen or estrogen and certain thromboembolic conditions. Swedish authorities noted decreased reporting of thromboembolic episodes when higher estrogen preparations were no longer prescribed. Careful epidemiological studies to determine degree of thromboembolic disease risk associated with progestogen-only OCs have not been done. Thromboembolic disease has been reported in women using these products, and they should not be considered free of excess risk. PERSISTENCE OF RISK. Two studies have suggested an increased risk may persist for 6 years after discontinuation of OC use for cerebrovascular disease and 9 years for MI; another study suggested the persistence of risk for subarachnoid hemorrhage. ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES. A large British prospective study estimated mortality rate per 100,000 women per year from circulatory system diseases for OC users and nonusers according to age, smoking habits, and duration of use. The overall annual excess death rate for OC users was estimated to be 20/100,000 (ages 15-34–5/100,000; ages 35-44–33/100,000; ages 45-49–140/100,000). Risk is concentrated in long-term users and in smokers, and may persist after OC discontinuation. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all occurred in women 35 or older. An update provided the following rates: ages 15-34–1/6700 for nonsmokers and 1/2000 for smokers; ages 45 and over–1/2500 for nonsmokers and 1/500 for smokers. Risk appeared to increase with parity, but not with duration of use. Until more women under 35 with continuous use for 5 or more years are available, it is not possible to assess relative risk for this age group. Data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. Estimates include combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease for OCs) plus risk attributable to pregnancy or abortion if the method fails (which varies with the effectiveness of the contraceptive method). The study concluded that mortality associated with all contraceptive methods is below that of childbirth, except for OCs in women over 40 who smoke. (Rates given for pill only/smokers for each age are for smokers as a class. For "heavy" smokers (more than 15 cigarettes a day), the rates would be about double; for "light" smokers (less than 15), about half.) The lowest mortality is with the condom or diaphragm backed up by early abortion. The study also concluded that OC users who smoke, especially over 30, have greater mortality risk than OC users who do not smoke.

Risk of thromboembolic and thrombotic disease associated with OCs increases with age after 30 and for MI is further increased by hypertension, hyperlipidemias, obesity, diabetes, or history of preeclamptic toxemia, and especially by smoking. The following chart gives a gross estimate of risk of death from circulatory disorders associated with OC use.

SMOKING HABITS AND OTHER PREDISPOSING CONDITIONS–RISK ASSOCIATED WITH USE OF OCs

Age	Below 30	30-39	40 +
Heavy smokers	C	B	A
Light smokers	D	C	B
Nonsmokers (no predisposing conditions)	D	C,D	C
Nonsmokers (other predisposing conditions)	C	C,B	B,A

A–Use associated with very high risk.
B–Use associated with high risk.
C–Use associated with moderate risk.
D–Use associated with low risk.

Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, discontinue OC immediately. A 4-6 fold increased risk of post-surgery thromboembolic complications has been reported in OC users. If feasible, discontinue OCs at least 4 weeks before surgery associated with increased risk of thromboembolism or prolonged immobilization. Before resuming OC after major surgery or bedrest, balance risks of post-surgery thromboembolic complications with contraceptive needs. Data suggest varicose veins substantially increase risk of superficial venous thrombosis of the leg, the risk depending on the severity of the varicosities. 2. *Ocular Lesions:* Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OC use. Discontinue OC if there is unexplained, sudden, or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions; and institute appropriate diagnostic and therapeutic measures. 3. *Carcinoma:* Long-term continuous administration of natural or synthetic estrogen in certain animals increases certain tumors, benign or malignant, such as breast, cervix, vagina, uterus, ovary, pituitary and liver. Certain synthetic progestogens, none currently in OCs, increase the incidence of mammary nodules, benign and malignant, in dogs. Several retrospective case-control studies reported an increased relative risk (3.1-13.9 times) associating endometrial carcinoma with the prolonged use of estrogens in postmenopausal women. One publication reported the first 30 cases submitted to the registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the adenocarcinomas found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding when OCs were first given, polycystic ovaries), nearly all occurred in women who had used sequential OCs, which are no longer marketed. No statistical association has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs, although individual cases have been reported. Studies have shown no increased risk of breast cancer to OC or estrogen users. One study found no overall increased risk of breast cancer in OC users but a greater risk was suggested for OC users with documented benign breast disease and for long-term (2-4 years) users. Another study found a history of breast cancer among grandmothers or aunts was significantly more frequent among breast cancer patients who had used an OC continuously for one or more years than among nonusers with breast cancer. One other study indicated an increasing risk of breast cancer in women taking menopausal estrogens, which increased with duration of follow-up. One author suggests that extended (over 6 years) OC use prior to first full term pregnancy was associated with a significant relative risk of breast cancer, and other studies suggested an increased risk of breast cancer with long-term OC use before age 25. A reduced occurrence of benign breast tumors in OC users has been well documented. One study reported malignant melanoma more frequently in OC users than controls and suggests an increased incidence of urinary tract and thyroid cancers. A prospective study of women with cervical dysplasia found an increase in severity and conversion to cancer *in situ* in OC users compared to nonusers. This became statistically significant after 3-4 years of use. Nonreversal of dysplasia within the first 6 months of pill use was suggested to predict progression after prolonged exposure. One study disclosed an increased risk of cancer of the cervix (largely carcinoma *in situ*) in OC users under 40, particularly those who had used OCs over 4 years. Two other studies suggested an increasing incidence of cervical neoplasms with longer duration of OC use. There have been other reports of microglandular hyperplasia of the cervix in OC users. One study reported an association between OC use and endocervical adenocarcinoma. In summary, there is no confirmed evidence from human studies of increased risk of cancer associated with OCs. Close clinical surveillance of all OC users is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, take appropriate diagnostic measures to rule out malignancy. Monitor OC users with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms with particular care. 4. *Liver Tumors:* Sudden severe abdominal pain or shock may be due to rupture and hemorrhage of a liver tumor. There have been reports associating benign or malignant liver tumors with short-term and long-term OC use. One study reported use of OCs with high hormonal potency and age over 30 may further increase risk of hepatocellular adenoma. Two studies relate risk with duration of use, risk being much greater after 4 or more years of use. Long-term OC users have an estimated annual incidence of hepatocellular adenoma of 3-4/100,000. Although an uncommon lesion, it should be considered in women presenting with an "acute abdomen". The tumor may cause serious or fatal hemorrhage. Patients with liver tumors have demonstrated variable clinical features which may make preoperative diagnosis difficult. Some cases presented because of right upper quadrant masses, while most had signs and symptoms of acute intraperitoneal hemorrhage. Routine radiological and laboratory studies may not be helpful. Liver scans may show a focal defect. Hepatic arteriography may be useful in diagnosing primary liver neoplasm. 5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring:* Use of female sex hormones—estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. Females exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1/1000 exposures or less. Although there is no evidence that OCs further enhance the risk of developing this type of malignancy, such OC users should be monitored with particular care. A high percentage of women exposed to diethylstilbestrol (30-90%) have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are a precursor of vaginal malignancy. Male children so exposed may develop urogenital tract abnormalities. Although similar data are not available on other estrogens, it cannot be presumed that they would not induce similar changes. Increased risk of congenital anomalies, including heart and limb defects, has been reported following use of sex hormones, including OCs, in pregnancy. One case-control study estimated a 4.7-fold increased relative risk of limb-reduction defects in infants exposed *in utero* to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some exposures involved only a few days of treatment. Data suggest risk of limb-reduction defects in exposed fetuses is somewhat less than 1/1000 live births. In a large prospective study, cardiovascular defects in children born to women who received female hormones, including OCs, during early pregnancy occurred at 18.2/1000 births, compared to 7.8/1000 for children not so exposed *in utero*. These results are statistically significant. A Welsh study found a statistically significant excess of neural tube defects among offspring of prior OC users (within 3 months) than among controls. The incidence of twin births may be increased for women who conceive shortly after discontinuing OC use. In the past, female sex hormones were used during pregnancy in an attempt to treat threatened or habitual abortion. There is evidence that estrogens are ineffective, and there is no evidence from well controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. If the patient has not adhered to the prescribed schedule, consider possible pregnancy at the first missed period (or 45 days from the last menstrual period if progestogen-only OCs are used) and discontinue OC use until pregnancy has been ruled out. For any patient who has missed two consecutive periods, rule out pregnancy before continuing the OC. If pregnancy is confirmed, tell the patient about potential risks to the fetus and discuss advisability of continuing the pregnancy. Women who discontinue OCs to become pregnant should use an alternate form of contraception for a period of time before attempting to conceive. A 3-month period is supported by a study suggesting increased frequency of neural tube defects in women impregnated during the first 3 months after cessation of OC use. Do not use progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding as a pregnancy test. 6. *Gall Bladder Disease:* Studies report increased risk of gall bladder disease in OC or estroge users. In one study, an increased risk appeared after 2 years of use and double after 4-5 years. In another study, an increased risk was apparent between 6 an 12 months. 7. *Carbohydrate and Lipid Metabolic Effects:* Because a decrease i glucose tolerance has been observed in a significant percentage of patients o OCs, prediabetic and diabetic OC users should be carefully observed. A increase in triglycerides and total phospholipids has been observed in OC user but its clinical significance is unknown. 8. *Elevated Blood Pressure:* An increas in blood pressure has been reported with OC use. Hypertension may occu within a few months of beginning OCs. In the first year of use, incidence o hypertension may be no higher in OC users than in nonusers. Incidence in user increases with exposure and in the fifth year of use is 2.5-3 times that in the first year. Age is strongly correlated with hypertension in OC users. Women wit a history of elevated blood pressure (hypertension), preexisting renal disease history of toxemia or elevated blood pressure during pregnancy, familial ten dency to hypertension or its consequences, or history of excessive weight gai or fluid retention during the menstrual cycle may be more likely to develo elevated blood pressure when given OCs and should be monitored closely. Eve though elevated blood pressure may remain within the "normal" range, closel watch elevations, particularly for women with other risk factors for cardiovascu lar disease or stroke. High blood pressure may or may not persist after OC discontinuation. 9. *Headache:* Discontinue OC and evaluate the cause of onset o exacerbation of migraine or development of a new pattern of headache which i recurrent, persistent, or severe. 10. *Bleeding Irregularities:* Breakthrough blee ing, spotting, and missed menses often make users discontinue OCs. In brea through bleeding, as in all cases of irregular bleeding from the vagina, conside nonfunctional causes. In undiagnosed persistent or recurrent abnormal bleedin from the vagina, use adequate diagnostic measures to rule out pregnancy o malignancy. If pathology has been excluded, time or another formulation ma solve the problem. While potentially useful in minimizing menstrual irregularity change to an OC with a higher estrogen content only if necessary since this ma increase the risk of thromboembolic disease. Women with a history of oligo menorrhea or secondary amenorrhea or young women without regular cycle may tend to remain anovulatory or to become amenorrheic after OC discontin uation. Women with these preexisting problems should be advised of this an encouraged to use other contraceptive methods. Post-use anovulation, possibl prolonged, may occur in women without previous irregularities. A higher inci dence of galactorrhea and of pituitary tumors (e.g., adenomas) has been asso ciated with amenorrhea in former users compared with nonusers. One stud reported a 16-fold increased incidence of pituitary prolactin-secreting tumor among patients with postpill amenorrhea when galactorrhea was present. 1 *Infertility:* There is evidence of impairment of fertility in women discontinuin OCs in comparison with other contraceptive methods, which appears to b independent of duration of use. While impairment diminishes with time, there i an appreciable difference in the results in nulliparous women for OC and othe groups 30 months after discontinuation of birth control. For parous women the difference is not apparent 30 months after cessation of contraception. One study suggests *Chlamydia trachomatis* infection, the leading cause of nongonococca P.I.D., is enhanced by OCs. 12. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in OC failure. In progestogen-only OC failures, the ratio of ectopic to intrauterine pregnancies is higher than in nonusers, since the drugs are more effective in preventing intrauterine than ectopic pregnancies. 13. *Breast Feeding:* OCs in the postpartum period may interfere with lactation by decreasing quantity and quality of breast milk. A small fraction of OC hormonal agents has been identified in milk of mothers receiving OCs. Effects, if any, on the breast-fed child have not been determined. If feasible, defer OC use until the infant has been weaned. **Precautions** *GENERAL* 1. Take a complete medical and family history before starting OCs. Pretreatment and periodic physical exams should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant lab tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may enlarge. 3. Observe patients with a history of psychic depression and discontinue OCs if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the OC and use an alternate method of contraception to determine whether the symptom is drug related. 4. OCs may cause some fluid retention. Prescribe with caution, and only with careful monitoring, in patients with conditions that might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma or cardiac, hepatic or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while using OCs. Discontinue OC if jaundice develops. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism that may result in a relative pyridoxine deficiency. The clinical significance is unknown. 8. Serum folate levels may be depressed by OC use. Since the pregnant woman is predisposed to folate deficiency and incidence of folate deficiency increases with increasing gestation, if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and related complications. 9. Advise the pathologist of OC use when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing OCs. For example: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. f. Increased phospholipids and triglycerides. g. Temporarily decreased glucose tolerance. 11. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist and temporary or permanent cessation of wear considered. DRUG INTERACTIONS. OCs may be less effective and there may be increased breakthrough bleeding from interactions with rifampin, isoniazid, ampicillin, tetracycline, neomycin, penicillin V, chloramphenicol, sulfonamides, nitrofurantoin, barbiturates, phenytoin, primidone, analgesics, tranquilizers, antimigraine preparations, antihistamines, and griseofulvin. OCs may alter effectiveness of such other drugs as oral anticoagulants, anticonvulsants, tricyclic antidepressants, antihypertensive agents (e.g., guanethidine), vitamins, hypoglycemic agents, tranquilizers, hypnotic preparations, theophylline, and beta blockers. Toxic liver effects have been reported with a combination of an OC and cyclosporin A. *CARCINOGENESIS* See Warnings. *PREGNANCY* Pregnancy category X. See Contraindications and Warnings. *NURSING MOTHERS* See Warnings. **Adverse Reactions** An increased risk of the following serious adverse reactions has been associated with OC use (see Warnings): thrombophlebitis, thrombosis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, mesenteric thrombosis, liver tumors, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis, Raynaud's disease, arterial thromboembolism. The following adverse reactions have been reported in OC users and are believed to be drug related: bleeding irregularities (breakthrough bleeding, spotting, missed menses during treatment, amenorrhea after treatment), gastrointestinal symptoms (nausea, vomiting, bloating, abdominal cramps, colitis), dysmenorrhea, infertility after discontinuance of treatment, edema, chloasma or melasma which may persist after drug is discontinued, breast changes (tenderness, enlargement, and secretion), intolerance to contact lenses, changes in corneal curvature (steepening), change in weight (increase or decrease), change in cervical erosion and cervical secretion, possible diminution of lactation when given immediately postpartum, cholestatic jaundice, migraine, increase in size of uterine leiomyomata, rash (allergic), mental depression, reduced tolerance to carbohydrates, vaginal candidiasis, prolactin-secreting pituitary tumors, chilblains. The following adverse reactions have been reported in OC users and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of hair, erythema multiforme, erythema nodosum, hemorrhagic eruptions, vaginitis, porphyria, impaired renal function, malignant nephrosclerosis (hemolytic uremic syndrome), malignant hypertension, acute renal failure. **Information for the Patient** (See Patient Package Insert).

Rev. 6/

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American Journal of obstetrics

A Standard in Progesterone Therapy

Provera® 10 MG TABLETS
(medroxyprogesterone acetate)

WARNING

THE USE OF PROGESTATIONAL AGENTS DURING THE FIRST FOUR MONTHS OF PREGNANCY IS NOT RECOMMENDED.

Progestational agents have been used beginning with the first trimester of pregnancy in an attempt to prevent habitual abortion or treat threatened abortion. There is no adequate evidence that such use is effective and there is evidence of potential harm to the fetus when such drugs are given during the first four months of pregnancy. Furthermore, in the vast majority of women, the cause of abortion is a defective ovum, which progestational agents could not be expected to influence. In addition, the use of progestational agents, with their uterine-relaxant properties, in patients with fertilized defective ova may cause a delay in spontaneous abortion. Therefore, the use of such drugs during the first four months of pregnancy is not recommended.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies including congenital heart defects and limb reduction defects. One study estimated a 4.7-fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 in 1000.

If the patient is exposed to PROVERA Tablets (medroxyprogesterone acetate) during the first four months of pregnancy or if she becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus.

INDICATIONS: Secondary amenorrhea; abnormal uterine bleeding due to hormonal imbalance in the absence of organic pathology such as fibroids or uterine cancer.

CONTRAINDICATIONS: Thrombophlebitis, thromboembolic disorders, cerebral apoplexy or patients with a past history of these conditions. Liver dysfunction or disease. Known or suspected malignancy of breast or genital organs. Undiagnosed vaginal bleeding. Missed abortion. Known sensitivity to medroxyprogesterone acetate. As a diagnostic test to pregnancy.

WARNINGS: 1. Immediately discontinue administration should any of the following thrombotic disorders occur or be suspected: thrombophlebitis, cerebrovascular disorders, pulmonary embolism, retinal thrombosis. **2.** Beagle dogs treated with medroxyprogesterone acetate developed mammary nodules some of which were malignant. Although nodules occasionally appeared in control animals, they were intermittent in nature; whereas the nodules in the drug treated animals were larger, more numerous, persistent, and there were some breast malignancies with metastases. Their significance with respect to humans has not been established. **3.** Discontinue medication pending examination if there is sudden partial or complete loss of vision, onset of proptosis, diplopia, or migraine. If papilledema or retinal vascular lesions occur, withdraw medication. **4.** Detectable amounts of progestin have been identified in the milk of mothers receiving the drug. The effect of this on the nursing infant has not been determined. **5.** Usage in pregnancy is not recommended (see Warning box). **6.** Three major studies in Great Britain and one in this country have shown a statistically significant association between thrombophlebitis, pulmonary embolism, cerebral thrombosis and embolism and the use of oral contraceptives. It has been estimated that users are several times as likely to undergo thromboembolic disease without evident cause as nonusers. The American study indicated that the risk did not persist after discontinuation, and it was not enhanced by long-continued administration.

PRECAUTIONS: A pretreatment physical exam should include special reference to breast and pelvic organs and a Papanicolaou smear. This drug may cause fluid retention; therefore, carefully observe patients with conditions influenced by fluid retention such as epilepsy, migraine, asthma, and cardiac or renal dysfunction. In irregular bleeding per vaginum, bear in mind nonfunctional causes and perform adequate diagnostic measures. Advise pathologist of therapy when submitting relevant specimens. Carefully observe patients with history of psychic depression and discontinue drug if serious depression recurs. Any possible influence of prolonged therapy on pituitary, ovarian, adrenal, hepatic, or uterine function awaits further study. Decreased glucose tolerance has been observed in a small percentage of patients on estrogen-progestin combinations; therefore, carefully observe diabetic patients receiving progestin therapy. Age constitutes no absolute limiting factor, although onset of climacteric may be masked. Because of the occasional occurrence of thrombotic disorders (thrombophlebitis, pulmonary embolism, retinal thrombosis, and cerebrovascular disorders) in patients taking estrogen-progestin combinations and since the mechanism is obscure, the physician should be alert to the earliest manifestation of these disorders. (See Patient Information for complete prescribing information.)

ADVERSE REACTIONS: Pregnancy: (see Warning box); **Breast:** rare reports of breast tenderness or galactorrhea; **Skin:** sensitivity reactions including pruritus, urticaria, edema and generalized rash; acne, alopecia and hirsutism in a few patients; **Thromboembolic Phenomena** including thrombophlebitis and pulmonary embolism.

The following adverse reactions have been observed in women taking progestins including medroxyprogesterone acetate: breakthrough bleeding; spotting; change in menstrual flow; amenorrhea; edema; change in weight; changes in cervical erosion and secretions; cholestatic jaundice; rash (allergic) with and without pruritus; mental depression; anaphylaxis and anaphylactoid reactions; pyrexia; insomnia; nausea and somnolence.

A statistically significant association has been demonstrated between use of estrogen-progestin combination drugs and the serious adverse reactions of thrombophlebitis, pulmonary embolism and cerebral thrombosis and embolism. Therefore, patients on progestin therapy should be carefully observed.

Although available evidence is suggestive, a relationship has been neither confirmed nor refuted for the association of the serious adverse reaction of neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been observed in patients receiving estrogen-progestin combination drugs: rise in blood pressure in susceptible individuals; premenstrual-like syndrome; changes in libido; changes in appetite; cystitis-like syndrome; headache; nervousness; dizziness; fatigue; backache; hirsutism; loss of scalp hair; erythema multiforme; erythema nodosum; hemorrhagic eruption; and itching. Therefore, observe patients on progestin therapy carefully.

The following laboratory results may be altered by the use of estrogen-progestin combination drugs: increased sulfobromophthalein retention and other hepatic function tests; coagulation tests (increase in prothrombin factors VII, VIII, IX and X); metyrapone test; pregnanediol determination; thyroid function tests (increase in PBI, and butanol extractable protein bound iodine and decrease in T^3 uptake values).

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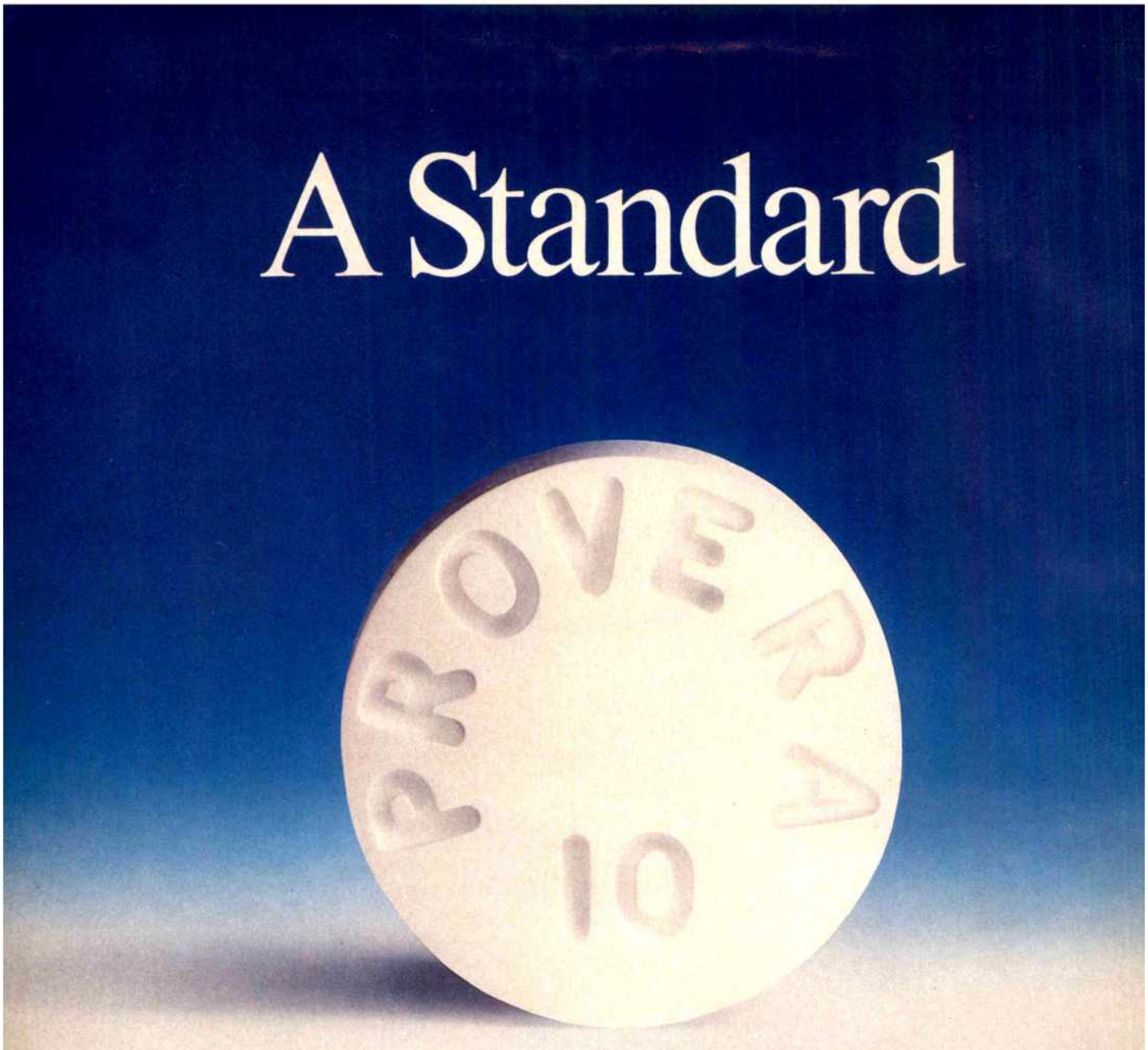

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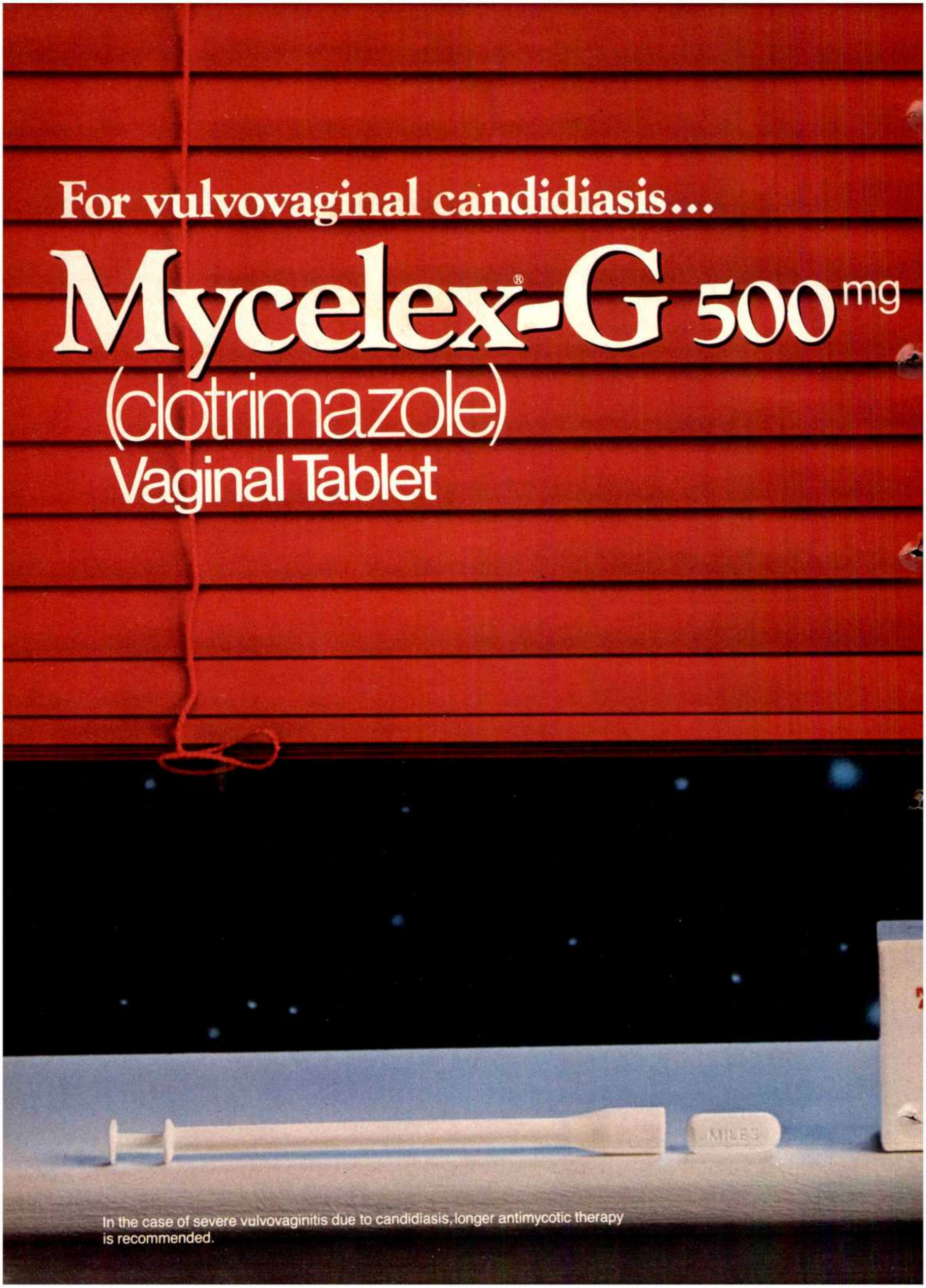

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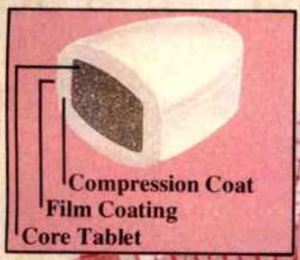

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Biotin, mg	0.05	16
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MINERALS		
Calcium, mg	200	15
Iodine, mcg	150	100
Iron,mg	60	333
Magnesium, mg	100	22
Copper, mg	2	100
Zinc, mg	15	100
Artificial color	none	
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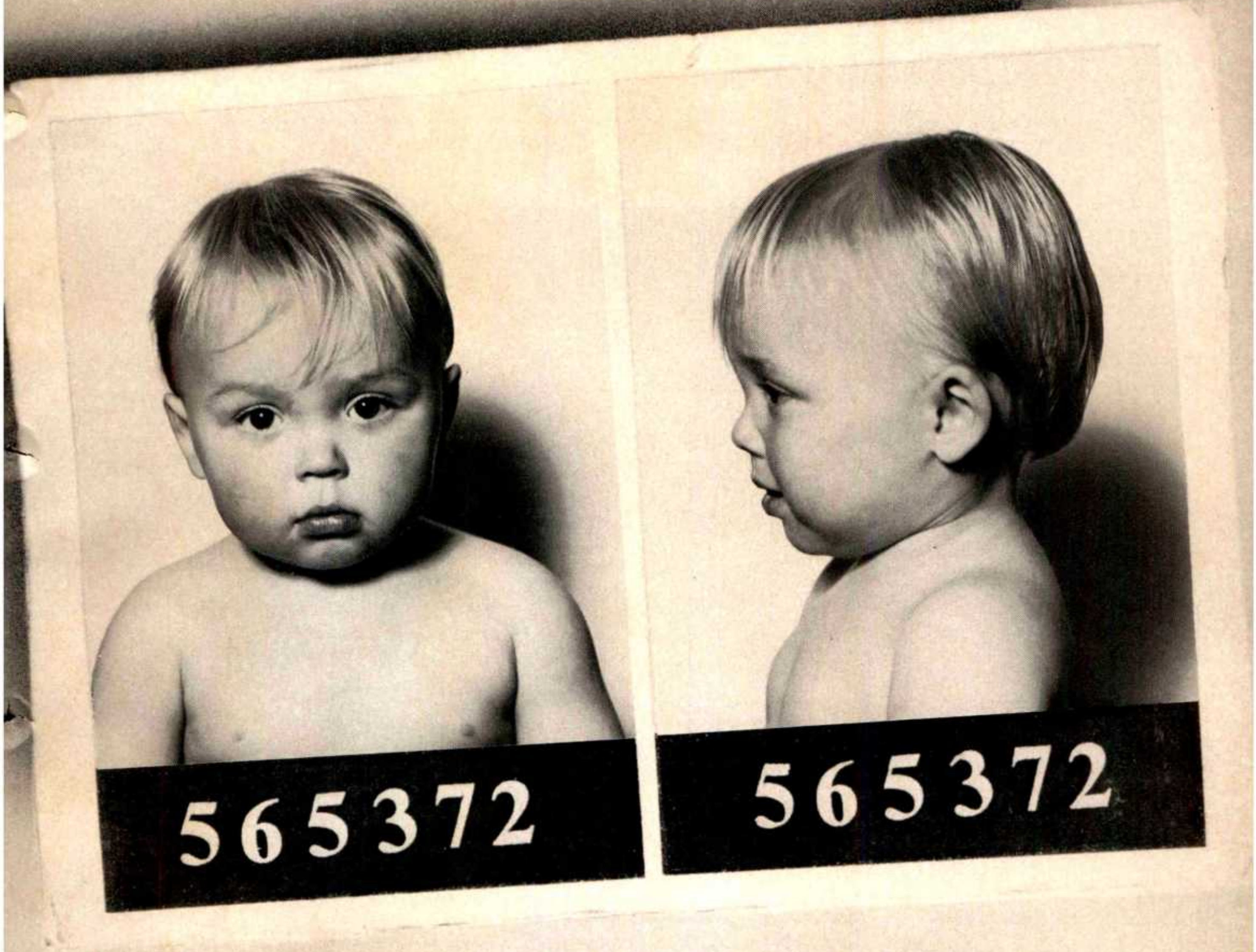

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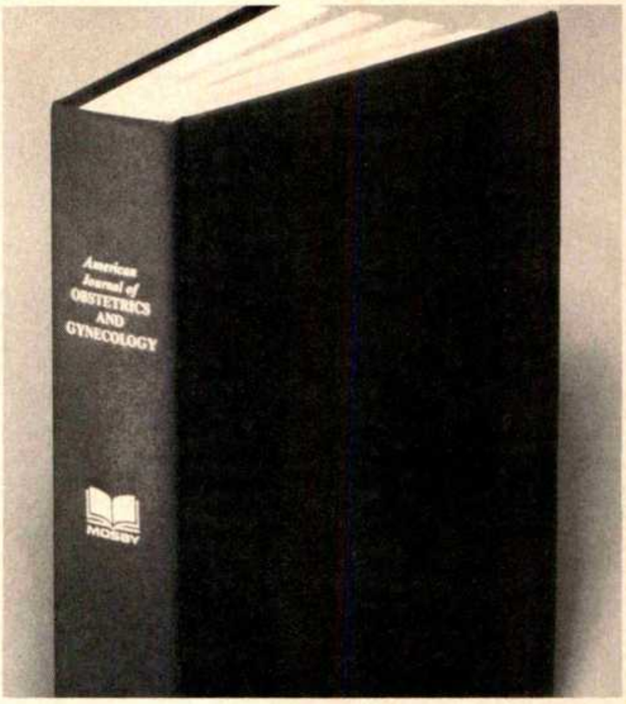

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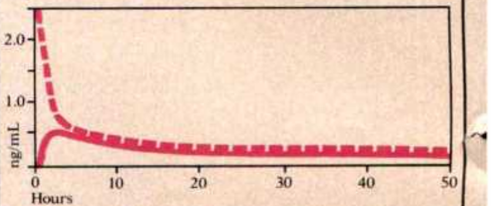

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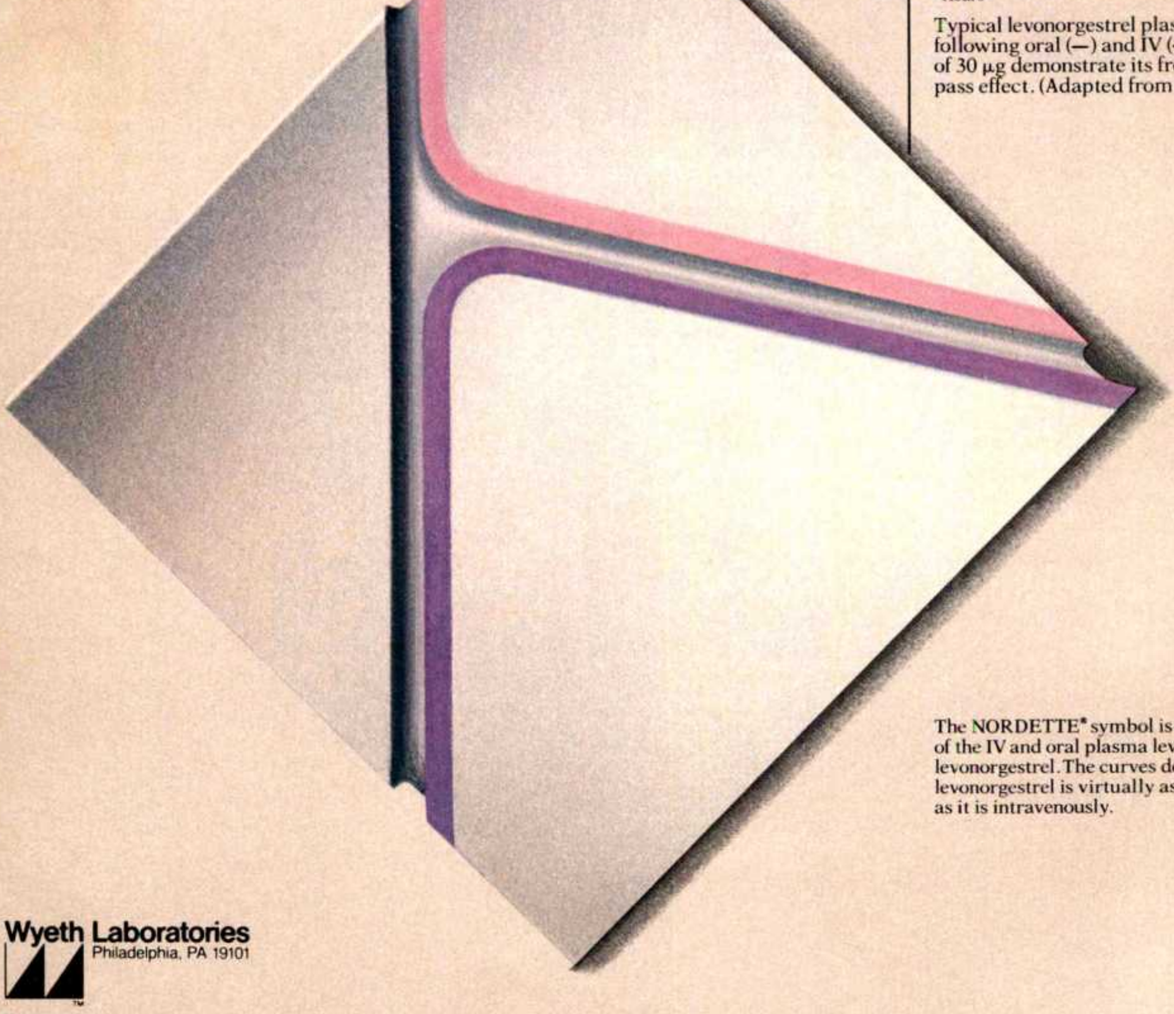

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With its prolonged half-life, levonorgestrel persists in the plasma for an extended period. Moreover, there is no clinically significant accumulation during repeated administration.

STEROID	DRUG PERSISTENCE (AS MEASURED BY MEAN ELIMINATION HALF-LIFE)
NORETHINDRONE	**7.5** hours[6]
LEVONORGESTREL	**11.4** hours[7]
ETHINYL ESTRADIOL	**10.0** hours[8]

And the clinical performance to back it up

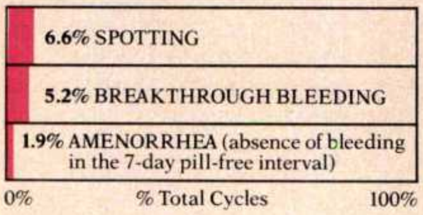
Levonorgestrel has established an impressive clinical record when used in combination with low doses of ethinyl estradiol in thousands of healthy young women.

High efficacy at an ultra low dose

In clinical use, low-estrogen OCs employing levonorgestrel such as NORDETTE® (each tablet contains 0.15 mg levonorgestrel with 0.03 mg ethinyl estradiol, Wyeth) have consistently demonstrated high efficacy and excellent cycle control.

Low incidence of common OC side effects*

In clinical studies involving 15,207 cycles of experience in more than 1,100 women, the levonorgestrel-containing formulation NORDETTE established this impressive record of patient acceptance and minimal cycle disturbance.



Minimal impact on blood lipid levels

Four independent investigations,[9-12] ranging from 3 to 12 months, offer thorough information on the long-term effect of NORDETTE on important lipid parameters. These controlled studies demonstrate the minimal impact NORDETTE has on total cholesterol, HDL cholesterol and LDL cholesterol. Some studies did not show a significant increase in triglycerides, while others did. No other OC in its class provides more comprehensive data.

INVESTIGATOR	(MONTHS)	TOTAL CHOLESTEROL	TRIGLYCERIDES	HDL CHOLESTEROL	LDL CHOLESTEROL
ÅHRÉN[9]	12	=	=	=	=
NASH[10]	3	=	▲	=	=
BERGINK[11]	3	=	▲	=	
BERGINK[12]	12	=			

▲ Increased = No Significant Change ☐ Not Tested

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*Serious as well as minor adverse reactions have been reported following the use of all oral contraceptives.

References:

1. Hümpel M, *et al: Contraception 17*:207-220, 1978.
2. Back DJ, *et al: Drugs 21*:46-61, 1981.
3. Orme M L'E: *Br J Clin Pharmacol 14*:31-42, 1982.
4. Newburger J, Goldzieher JW: *Contraception 32*:33-34, 1985.
5. Back DJ, *et al: Clin Pharmacol Ther 24*:448-453, 1978.
6. Forchielli E: *Norethindrone, The First Three Decades.* Palo Alto, Syntex Laboratories, 1978, p 48.
7. Back DJ, *et al: Contraception 23*:229-239, 1981.
8. Goldzieher JW, Dozier TS, de la Pena A: *Contraception 21*:1-16, 1980.
9. Åhrén T, *et al: Contraception 24*:451-468, 1981.
10. Nash AL, *et al: Med J Austr 2*:277-281, 1979.
11. Bergink EW, *et al: Contraception 30*:61-72, 1984.
12. Bergink EW, *et al: Contraception 25*:477-485, 1982.

See important information on adjacent page.

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each tablet contains 0.15 mg levonorgestrel with 0.03 mg ethinyl estradiol, Wyeth.

WITH LEVONORGESTREL...
THE VIRTUALLY 100% BIOAVAILABLE PROGESTOGEN

IN BRIEF:

Indications and Usage —NORDETTE® is indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OC's) as a method of contraception.

Contraindications —OC's should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during use of OC's or other estrogen-containing products.

Warnings

> **Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.**
> The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems*—An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION (MI)—An increased risk of MI associated with the use of OC's has been reported, confirming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia) the higher the risk of developing MI, regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important factor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; quite likely the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse-reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose of estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the U.S.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OC's according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), risk being concentrated in older women, in those with long duration of use and in cigarette smokers. It was not possible, however, to examine interrelationships of age, smoking, and duration of use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates of risk of death for each method include combined risk of contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OC's) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effectiveness of method. The study concluded that mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OC's in women over 40 who smoke. Lowest mortality is associated with condom or diaphragm backed up by early abortion. Risk of thromboembolic and thrombotic disease associated with OC's increases with age after about 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclamptic toxemia, and especially cigarette smoking. Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A 4- to 6-fold increased risk of postsurgery thromboembolic complications has been reported in OC users. If feasible, OC's should be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Britain involving cerebrovascular disease and another in the U.S. concerning MI suggest an increased risk of these conditions in users of OC's persists after discontinuation of the OC's. In the British study, risk of cerebrovascular disease remained elevated in former OC users for at least 6 years after discontinuation. In the U.S. study, increased risk of MI persisted for at least 9 years in women 40 to 49 years old who had used OC's for 5 or more years. Findings in both studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions*—There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma*—Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in OC's, have been noted to increase incidence of mammary nodules, benign and malignant, in dogs. In humans, 3 case-control studies have reported an increased risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OC's. Of cases found in women without predisposing risk factors (e.g., irregular bleeding at the time OC's were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No evidence has been reported suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only OC's. Several studies have found no increase in breast cancer in women taking OC's or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women on OC's, found an excess risk in subgroups of OC users with documented benign breast disease. Reduced occurrence of benign breast tumors in users of OC's has been well documented. In summary, there is at present no confirmed evidence from human studies of increased risk of cancer associated with OC's. Close clinical surveillance of all women on OC's is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use OC's.

4. *Hepatic Tumors*—Benign hepatic adenomas have been found to be associated with use of OC's. One study showed that OC's with high hormonal potency were associated with higher risk than lower potency OC's. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. Two studies relate risk with duration of use of OC's, the risk being much greater after 4 or more years' use. While hepatic adenoma is rare, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women on OC's. Relationship of these drugs to this type of malignancy is not known.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring*—Use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have increased risk of developing in later life a form of vaginal or cervical cancer ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OC's further enhance risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OC's. Furthermore, 30 to 90% of such exposed women have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with use of other estrogens, it cannot be presumed they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with use of sex hormones, including OC's, in pregnancy. One case-control study estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OC's, hormonal withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some exposures involved only a few days. Data suggest that risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OC's. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OC's is unknown. It is recommended that, for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time of first missed period, and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation of the pregnancy should be discussed. It is also recommended that women who discontinue OC's with intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this. The administration of progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease*—Studies report increased risk of surgically confirmed gallbladder disease in users of OC's and estrogens. In one study, increased risk appeared after 2 years' use and doubled after 4 or 5 years' use. In one of the other studies, increased risk was apparent between 6 and 12 months' use.

7. *Carbohydrate and Lipid Metabolic Effects*—Decrease in glucose tolerance has been observed in a significant percentage of patients on OC's. For this reason, prediabetic and diabetic patients should be carefully observed while on OC's. Increases in triglycerides and total phospholipids have been observed in patients on OC's. Clinical significance of this finding remains to be defined.

8. *Elevated Blood Pressure*—Increase in blood pressure has been reported in patients on OC's. In some women, hypertension may occur within a few months of beginning OC's. In the 1st year of use, prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. Prevalence in users increases, however, with longer exposure, and in the 5th year of use is 2½ to 3 times the reported prevalence in the 1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OC's. Hypertension that develops as a result of taking OC's usually returns to normal after discontinuing the drug.

9. *Headache*—Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of OC's and evaluation of the cause.

10. *Bleeding Irregularities*—Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing OC's. In breakthrough bleeding, as in all cases of irregular vaginal bleeding, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another OC may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuing OC's. Women with these preexisting problems should be advised of this possibility and encouraged to use other methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy*—Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding*—OC's given in the postpartum period may interfere with lactation and decrease quantity and quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the milk of mothers on OC's; effects, if any, on the breast-fed child have not been determined. If feasible, defer OC's until infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OC's. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests. As a general rule, OC's should not be prescribed for longer than 1 year without another physical examination.

2. Under influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size.

3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop OC's and use an alternate method to try to determine whether the symptom is drug-related.

4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency.

5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OC's. If jaundice develops, OC's should be discontinued.

6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution.

7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance is undetermined.

8. Serum folate levels may be depressed by OC's. Since the pregnant woman is predisposed to development of folate deficiency and incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OC's, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency.

Information for the Patient—See Patient Package Labeling.

Laboratory Tests—1. The pathologist should be advised of OC therapy when relevant specimens are submitted.

2. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OC's.

a. Increased sulfobromophthalein retention.

b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.

c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered.

d. Decreased pregnanediol excretion.

e. Reduced response to metyrapone test.

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin, and tetracycline.

Carcinogenesis, Mutagenesis, Impairment of Fertility—See Warnings section #3, 4, and 5 for information on carcinogenesis, mutagenesis, and impairment of fertility.

Pregnancy—Category X. See Contraindications, Warnings.

Nursing Mothers—See Warnings. Because of potential for adverse reactions in nursing infants from oral contraceptive tablets, a decision should be made whether to discontinue the drug, taking into account the importance of the drug to the mother.

Adverse Reactions—An increased risk of these serious adverse reactions has been associated with the use of OC's (see Warnings): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies. There is evidence of an association between the following conditions and use of OC's, although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients on OC's and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatment; edema, chloasma or melasma which may persist; breast changes: tenderness, enlargement, and secretion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses.

The following adverse reactions have been reported in users of OC's, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OC's by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

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CLINICAL SECTION

Clinical Opinion

A hypothesis concerning the general basis of organogenetic congenital anomalies

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Evidence supports the idea that it is the degree of metabolic imbalance present in diabetic gravid women during the period of organogenesis that accounts for organogenetic congenital defects. In light of the proved and inferred metabolic instability occurring during early pregnancy, we propose that metabolic imbalances may occur that result in organogenetic congenital defects in offspring of apparently normal gravid women. (AM J OBSTET GYNECOL 1987;157:1-4.)

Key words: Organogenesis, birth defect, diabetes mellitus

Birth defects continue to be an important cause of morbidity and death. Among these we refer to those malformations that arise during the period of organogenesis (the second to eighth weeks after conception) as organogenetic congenital anomalies. Their cause is largely unknown. Based on a composite of several lines of information that bear on pregnancies in normal and diabetic gravid women, the authors hypothesize a possible common underlying pathogenic mechanism for anomalous organogenesis and propose research that may clarify the issue. Of course the ultimate goal of this exercise is to put the theories to use in diminishing the incidence and severity of birth defects, especially organogenetic anomalies.

Diabetes mellitus appears to contribute to organogenetic congenital defects by inducing the diabetic state

The disease diabetes mellitus leads to a syndrome, the diabetic state, that includes aberrations of carbohydrate, lipid, and protein metabolism. This is most commonly recognized as a relative or absolute glucose intolerance, elevated blood glucose levels, and ketoacidosis. It has long been known that, in addition to other metabolic problems and progression of the disease, diabetic gravid women have a twofold or more increased incidence of fetal wastage and organogenetic congenital anomalies among their offspring.[1] However, this is not the case for offspring of diabetic fathers and normal mothers[2]; in fact, fetal chromosomal abnormalities or other underlying maternal causes of fetal wastage are not considered to be in excess.[3] Furthermore, although HLA antigen studies of diabetic mothers showed no correlation with the occurrence of anomalies in children,[3] a large retrospective study of mothers who had offspring with anomalies showed a clear excess of subsequent adult onset diabetes mellitus.[4]

Despite the high incidence of congenital anomalies in offspring of diabetic mothers, there is not a "diabetes specific" anomaly, nor are the organogenetic congenital

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defects among offspring of diabetic mothers of an unusual nature.[1, 2] They frequently occur in multiple systems, including congenital heart disease, skeletal disorders, neural tube defects, and gastrointestinal disorders.[2] Although the rare "caudal regression syndrome" has been mentioned as diabetes specific, its occurrence is not restricted to the diabetic pregnancy and its rarity confounds interpretation of apparent excesses in diabetic pregnancies.[5] The epidemiology of congenital defects and pregnancy wastage in diabetic pregnancies is fragmentary; however, the diabetic pregnancy appears to exert an additive effect on the incidence of neural tube defects in a known high-risk group (A. Milunsky, personal communication). Furthermore, although it has been established that the incidence of congenital anomalies among diabetic gravid women is increased when glucose control is poor,[6] the effect of aberrations in lipid and protein metabolism on development of malformations has not been shown.[7]

Despite considerable progress in our understanding of the basis of diabetes mellitus and its treatment, we have not affected the incidence of congenital anomalies in offspring of diabetic mothers by stringent metabolic control of the already pregnant diabetic woman.[1] However, since in almost all of these women the diagnosis of pregnancy was made well into, or even beyond, the period of organogenesis, strict diabetic management was not exercised during the risk period for the development of organogenetic congenital anomalies.[7] Early reports indicate that this situation is probably corrected when strict metabolic control is established before pregnancy.[8]

We are thus faced with the following: (1) diabetes in pregnancy carries a variably increased risk of fetal wastage and organogenetic congenital malformations, (2) the risk is related to the diabetic state, and (3) there are no "diabetes specific" anomalies. Accordingly, it appears that these developmental abnormalities may be more related to insults such as the metabolic derangements of the diabetic state than to the disease diabetes mellitus per se. The idea that epigenetic maternal factors can play a role, presumably via nutrition of the embryo-fetus, in gestational development is not new; in fact, Freinkel[7] and Bennett et al.,[2] in comparing data from several sources of diabetic and prediabetic pregnancies, have independently reached the same opinion as we regarding the importance of the diabetic state to the outcome of diabetic pregnancies. In reviewing these matters further, we have come to believe that since metabolic derangements similar to those seen in diabetic gravid women may occur in nondiabetic pregnant women, it is possible that subclinical or unnoticed metabolic derangements occurring during the embryonic period in otherwise normal pregnancies could also pose a teratogenic risk. Let us briefly review some experimental data that support this hypothesis.

The diabetic state and other teratogenic influences

Because the teratogenic risk seems to be related to the severity of the diabetic state, it is important to know what elements of the diabetic state cause teratogenesis. Experimentally induced hyperglycemia has caused organogenetic congenital anomalies, and similar effects have been induced in vitro by treatment during organogenesis, including excess glucose, hyper- or hypooxygenation, hyperthermia, trypan blue injection, ketones, heavy metals, and antisera to yolk sac.[9] This picture indicates that a common locus that is sensitive to many factors could be the source of organogenetic anomalies. Considerable evidence indicates that this vulnerable locus is exposed during the period of organogenesis, a time that is particularly sensitive and critical to maintenance of the embryo's life and the fulfillment of its primordial developmental program. Studies with intact rat embryos and cultured rat conceptuses indicate that the yolk sac may harbor a critical locus involved in these developmental misadventures.[9] Under abnormal conditions such as described above, the yolk sac fails to form normally. Characteristically, this includes morphologic abnormalities, abnormalities of its transport and protein synthetic function, and the failure to form the vitelline circulation. The effect of hyperglycemia on the yolk sac is not caused by ℓ-glucose and is not due to increased osmolality. Yolk sac failure can deprive the embryo of gaseous and nutrient transfer (asphyxia), impair proper development of the circulatory, gastrointestinal, respiratory, and nervous systems, and detrimentally effect the generation of primordial germ cells. In support of this possibility, our studies on experimental rat models with hyperglycemia used to induce anomalies have indicated that arachidonic acid supplementation that prevents teratogenesis is associated with maintenance of cellular membrane integrity and the development of normal vitelline vessels.[10] In these embryos, precocious neuropil development[11] is also prevented by arachidonic acid supplementation (unpublished observations). Although the experimental work on the role of the yolk sac is promising, it has not ruled out other, non-yolk sac–dependent developmental effects in specific organs. As well, there are apparent but poorly understood differences in yolk sac anatomy between species, so the ultimate proof of the importance of yolk sac failure in human pregnancies requires studies in primates (see below). Because of this, the few available anecdotal reports that support the role of the yolk sac in human teratogenesis are of particular interest.[12]

Pregnancy and pregnancy-like states as contributors to abnormal metabolism

Pregnancy wastage and organogenetic defects have their origin during the first days of pregnancy when the embryo has specific and changing energy requirements. For example, the work of Freinkel et al.[13] using glucose epimers indicates that blockage of glycolysis may have embryotoxic effects. Much of the program of organogenesis is laid down during the first 25 days of embryo life; this includes the period in which the secondary or provisceral* yolk sac is prominent. Although it is critical to understand maternal and fetal metabolism during this period, it remains an area of almost complete ignorance in humans. To be sure, pregnancy and pregnancy-like states have been shown to effect glucose homeostasis and lipid and amino acid metabolism. The metabolic status of women in the second and third trimesters of pregnancy have been most widely studied and have been described as consistent with "facilitated anabolism" and "accelerated starvation."[7] However, a paucity of information exists regarding metabolic homeostasis in the first trimester of pregnancy, and even less is known during the specific periods of implantation and organogenesis (weeks 1 to 2 and 2 to 8, respectively). That changes in metabolism could be present is suggested by previous reports that demonstrate that estrogen/progestin contraceptives cause glucose intolerance and lipid abnormalities after brief periods of administration[14] and by studies in nonpregnant women that suggest that the luteal phase may be characterized by deterioration in metabolic homeostasis.[15]

Thus it is reasonable to consider early pregnancy as a time of metabolic instability when extremes of locally formed and circulatory nutrients and metabolites could be presented to the developing conceptus. There is evidence, some of which is described above, that the embryo is dependent on its milieu and would be sensitive to such changes. However, there is a remarkable dearth of evidence regarding this critical period in human and subhuman primates. This lack of information becomes ever-more conspicuous when one considers the possibility that early metabolic abnormalities could contribute to embryo wastage and organogenetic anomalies in offspring of normal and diabetic mothers.

Evaluating the hypothesis

We have presented a hypothesis that is based on a collection of diverse observations in animals and humans. Its validity is currently unknown and should be tested. Data should be generated to establish the metabolic status of periconceptual diabetic and nondiabetic women. The occurrence during this time of metabolic alterations in association with metabolic, dietary, environmental, and xenobiotically induced stresses should be documented. The embryologic sites(s) and mechanism(s) of organogenetic defects related to these metabolic stresses should be defined, and the mechanism of the protective effects of agents such as arachidonic acid should be determined. In addition, although our hypothesis arises from, and is illustrated by, the extremes of developmental abnormalities, embryo wastage and organogenetic congenital anomalies, similarly based but more subtle anatomic or morphologic defects may underlie functional abnormalities among apparently normal newborns.[11] A case in point is the recent evidence that peripartum factors cannot explain most cases of cerebral palsy,[16] which drives our attention toward the period of organogenesis as a possible setting for abnormalities of a smaller scale, which through their effect on the developmental program could have far-reaching functional repercussions. Some of the necessary studies must be performed by observing human pregnancies; however, others will require the refinement of animal models, including the development of subhuman primate models.

A clearer understanding of the etiology of congenital malformations awaits the performance of these studies. Hopefully this will lead to strategies for prevention and treatment of pregnancy wastage and fetal malformations.

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REFERENCES

1. Cousins L. Congenital anomalies among infants of diabetic mothers: etiology, prevention, and prenatal diagnosis. AM J OBSTET GYNECOL 1983;147:333.
2. Bennett PH, Webner C, Miller M. Congenital anomalies and the diabetic and prediabetic pregnancy. In Elliott K, O'Connor M, eds. Pregnancy, metabolism, diabetes and the fetus, Ciba Foundation Symposium 63. New York: Excerpta Medica, 1979:207-18.
3. Simpson JL, Elias S, Martin AO, Palmer MS, Ogata ES,

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*Since mammals have miolecithal eggs (i.e., containing meager quantities of yolk), the perception that the yolk sac is merely a nutritional storehouse has hindered an acceptance of the yolk sac as an important entity in mammalian embryogenesis. Nothing could be further from the truth, and we have therefore adopted the term "provisceral yolk sac" in place of the descriptive term "secondary yolk sac," to distinguish this embryonic organ for its critical role as the progenitor of organ systems in the fetus. Thus in the scheme of embryogenesis the primary yolk sac gives rise to the provisceral yolk sac, which becomes internalized in the embryo, budding off the spherical secondary yolk sac, which remains joined by the vitillene duct to the internalized portion of the provisceral yolk sac.

Radvany RA. Diabetes in pregnancy, Northwestern University series (1977-1981) I. Prospective study of anomalies in offspring of mothers with diabetes mellitus. AM J OBSTET GYNECOL 1983;146:263.

4. Navarrete VN, Rojas CE, Alger CR, Paniagua HE. Subsequent diabetes in mothers delivered of a malformed infant. Lancet 1970;2:993.
5. Blumel J, Evans EB, Eggers GW. Partial and complete agenesis or malformation of the sacrum with associated anomalies. J Bone Joint Surg 1959;41:497.
6. Miller E, Hare JW, Cloherty JP, et al. Elevated maternal hemoglobin AIC in early pregnancy and major congenital anomalies in infants of diabetic mothers. N Engl J Med 1981;304:1331.
7. Freinkel N. Of pregnancy and progeny. Diabetes 1980; 29:1023.
8. Fuhrmann K, Reiher H, Semmler K, Fischer F, Fischer M, Glockner E. Prevention of congenital malformations in infants of insulin-dependent diabetic mothers. Diabetes Care 1983;6:219.
9. Pinter E, Reece EA, Leranth CZ, et al. Yolk sac failure in embryopathy due to hyperglycemia. An ultrastructural analysis of the yolk sac differentiation associated with embryopathy in rat conceptuses under hyperglycemic conditions. Teratology 1986;33:363.
10. Pinter E, Reece EA, Leranth CZ, et al. Arachidonic acid prevents hyperglycemia-associated yolk sac damage and embryopathy. AM J OBSTET GYNECOL 1986;155:691.
11. Reece EA, Pinter E, Leranth CZ, et al. Ultrastructural analysis of malformations of the embryonic neural axis induced by hyperglycemic conceptus culture. Teratology 1986;32:363.
12. Crooij MG, Westhuis M, Shoemaker J, Eralto N. Ultrasonographic measurement of the yolk sac. Br J Obstet Gynecol 1982;89:931.
13. Freinkel N, Lewis NJ, Akazawa S, Roth SI, Gorman L. The honeybee syndrome—implications of the teratogenicity of mannose in rat-embryo culture. N Engl J Med 1984;310:223.
14. Spellacy WN. A review of carbohydrate metabolism and the oral contraceptives. AM J OBSTET GYNECOL 1969; 104:448.
15. Jarret RJ, Graver HJ. Changes in oral glucose tolerance during the menstrual cycle. Br Med J 1968;2:528.
16. Nelson KB, Ellenberg JH. Antecedents of cerebral palsy. N Engl J Med 1986;315:81.

Technical aspects of intravascular intrauterine transfusions: Lessons learned from thirty-three procedures

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Our technique for performing intravascular transfusions is described and four failed attempts are presented. The appropriate choice of needle, insertion site, and premedication are discussed. A rationale for intravenous fetal muscle blockade in selected cases is presented and a transfusion setup designed to minimize movement of the needle hub is described. Finally, the judicious use of intraperitoneal transfusions in some cases is suggested. (AM J OBSTET GYNECOL 1987;157:4-9.)

Key words: Intrauterine intravascular transfusion, Rh isoimmunization, fetal therapy

Several reports have attested to the usefulness of performing intrauterine transfusions intravascularly rather than by the intraperitoneal route. The advantages of transfusing directly into the fetal intravascular space are that the antigenic status of the fetal blood can be confirmed, pretransfusion and posttransfusion hematocrit values can be measured, and the transfused red cells are not dependent on lymphatic transport from the fetal peritoneal cavity for entry into the intravascular space. Blood has been transfused intravascularly by the fetoscopic approach or by percutaneous ultrasonic guidance into a cord vessel, a vessel within the liver, or even directly into the fetal heart.

During the past 18 months our group has used the percutaneous ultrasonically guided approach in 33 attempted transfusions. Twenty-nine (88%) of these attempts were technically successful. Details of the first 18 procedures in this series have previously been published.[1] The purpose of this report is to describe our technique in detail, present the four failed attempts, and discuss the lessons we have learned from our experience.

The technique

Our technique for performing an intravascular transfusion in utero is as follows: An ultrasonographic examination is performed before any medication is ad-

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ministered to the patient to locate the site of the cord insertion into the placenta and determine the precise lie of the fetus. The patient or fetus may have to be manipulated considerably to maximize exposure of the target vessel. An intravenous infusion is started and the patient is premedicated with a narcotic, a tranquilizer, an antiemetic, a tocolytic agent, and an antibiotic. The maternal abdomen is prepared and draped and, by sterile technique, a linear array transducer is used to locate the target vessel as close to the cord root as possible. The depth from the maternal skin surface at the needle entry site to the center of the vessel's lumen is then measured. A right-handed operator holds the transducer in his or her left hand and ultrasonically follows the course of the needle tip, which is introduced with the right hand. The needle is advanced slowly under direct visualization until the vessel lumen is entered. The needle's stylet is then removed and a 1 ml sample of blood is aspirated for cell size analysis and hematocrit determination. After it has been verified that a fetal vessel has been entered and a transfusion is indicated, the total volume of packed red blood cells estimated to raise the hematocrit into the normal range is calculated. Washed, irradiated, antigen–negative packed red blood cells with a known hematocrit are then administered directly through the needle. Flow of the transfused blood can be followed ultrasonically during the procedure. This is easier to see if the umbilical vein is entered but flow through the arteries can be visualized as well. The fetal cardiac rate is monitored ultrasonically throughout the transfusion by intermittently directing the transducer toward the fetal heart or a visibly pulsating artery. After transfusing approximately 75% of the estimated volume that will be required, another sample of fetal blood is aspirated to determine the hematocrit. If the value obtained is consistent with the calculated estimate, the remainder of the transfusion is completed and a final sample is aspirated before the needle is withdrawn. On the other hand, if the hematocrit value obtained midway through the procedure is very different from the anticipated value, the tip of the needle must be checked to be certain that it is still within a fetal vessel. If the needle has been dislodged it is readjusted and the process begun anew. However, demonstration that the tip is seated correctly requires recalculation of the amount of additional blood to be transfused. When the final hematocrit is documented to be within the desired range, the transfusion needle is withdrawn under direct ultrasonic visualization. The transfusion site is then observed for evidence of prolonged bleeding or hematoma formation. When all bleeding has stopped and the fetal heart rate is observed to be within the normal range the patient is transferred to the delivery floor for observation and external monitoring overnight. The following morning a scan is repeated at which time the fetus and transfusion site are carefully reexamined. If both the fetus and mother are stable the patient is then discharged.

Failed attempts

Case 1. K. R. was referred to our unit at 29½ weeks' gestation with signs of fetal hydrops secondary to Kell isoimmunization. Ultrasonographic assessment confirmed the presence of moderate fetal ascites and a small pericardial effusion. The placenta was located on the posterior uterine wall, and its cord insertion site initially could not be visualized. Therefore a needle was placed into an umbilical vessel at its entry site into the fetal abdomen. After determining that the hematocrit was 11%, the needle tip became displaced as a result of fetal movement. A second needle was then introduced into the umbilical vein at the cord insertion site into the placenta. This needle also became dislodged because of fetal movement. Further attempts at intravascular transfusion were then abandoned. Because of concern about possible blood loss secondary to the attempted procedures in this severely anemic fetus, the patient was delivered by immediate cesarean section. The newborn weighed 1470 gm and had a cord hematocrit of 10%. The infant required a total of two double-volume exchange transfusions within the first 4 days after birth and was subsequently discharged in good condition.

Case 2. D. C., a 220-pound woman, was referred to our unit at 22½ weeks' gestation with the diagnosis of fetal hydrops secondary to severe Rh isoimmunization. Her last pregnancy had ended in a stillbirth from fetal hydrops at 28 weeks. Ultrasonographic assessment confirmed the presence of fetal hydrops with a moderate degree of ascites and scalp edema. The placenta was in an anterior position. Percutaneous umbilical blood sampling revealed the fetal hematocrit to be 11%. A transfusion of 30 ml of packed red cells raised the hematocrit value to 35%. One week later, increasing fetal ascites was noted and a second intravascular transfusion was performed. This procedure was technically more difficult than the first one, primarily because of a uterine contraction that dislodged the needle tip. This factor, in association with the patient's obesity, necessitated the use of a longer needle, which resulted in successful reinsertion and made it possible to perform the transfusion. The hematocrit value was increased from 14% to 45% after administration of 30 ml packed red cells. The third procedure was scheduled 2 weeks later. Multiple attempts at gaining entry into the umbilical vessels at both the placental and abdominal insertion sites were technically unsuccessful. Therefore an intraperitoneal transfusion of 60 ml packed red cells was administered. Fetal bradycardia occurred intermittently during the intraperitoneal transfusion, although cardiac activity was normal at the conclusion of the procedure. No evidence of bleeding from fetal puncture sites or hematoma formation was noted on ultrasonographic inspection. Eight hours after the procedure death in utero occurred. The stillborn infant weighed 840 gm, and the cord hematocrit was 12%. Autopsy confirmed the presence of hydrops but there was no evidence to suggest injury to the cord, placenta, or fetal organs from the transfusion procedures.

Case 3. M. B., both of whose previous pregnancies had resulted in perinatal deaths from severe Rh isoimmunization at 28 and 25 weeks, underwent an elective percutaneous umbilical blood sampling procedure at 21 weeks. The placenta was on the left lateral wall of the uterus with the cord insertion located only 4 cm from the maternal anterior abdominal skin surface. No evidence of fetal hydrops was observed, but mild polyhydramnios was present. A 22-gauge, 9 cm needle was introduced easily into the cord root and a sample of aspirated fetal blood had a hematocrit of 30%. This blood was subsequently shown to be O Rh positive with a 4+ direct Coombs test. Despite the relatively good hematocrit value, it was decided to prophylactically administer some packed red cells. However, during the attachment of the intravenous tubing for the transfusion, the needle tip became dislodged from the vessel lumen. Multiple attempts to reenter the vessel were unsuccessful and a subchorionic collection of blood subsequently obscured visualization of the cord root. It was therefore decided to discontinue further efforts. Two weeks later this patient underwent the first of a series of five totally uneventful intravascular transfusions. No evidence of the subchorionic hematoma created during the first procedure was detectable on any of these subsequent occasions. She was electively delivered by repeat cesarean section at 36½ weeks. The neonate weighed 2570 gm, had an initial hematocrit of 38%, and required only a single exchange transfusion.

Case 4. A. D. was referred to our unit at 24 weeks with a history of severe Rh isoimmunization. Her most recent three pregnancies had resulted in stillbirths secondary to erythroblastosis fetalis. The placenta was in a posterior position. No evidence of hydrops was noted but mild polyhydramnios was present. An intravascular transfusion was performed uneventfully that raised the fetal hematocrit from 14% to 34%. A second transfusion was performed 2 weeks later. The cord root was entered with a 22-gauge, 11 cm needle, and a sample of aspirated blood had a hematocrit of 21%. Because of fetal movement the tip became dislodged from the vessel lumen and was reinserted with some technical difficulty. After 17 ml packed cells was transfused the needle was again dislodged by a fetal kick. No further attempts to reenter the vessel were made and a final hematocrit was not obtained. Ten days later a third intravascular transfusion was performed. A 22-gauge, 11 cm needle was used that became dislodged once during the transfusion but was easily reintroduced, and the transfusion proceeded uneventfully. A fourth procedure was attempted 2½ weeks later. The gestational age was 30 weeks and no evidence of hydrops was noted. A 20-gauge, 11 cm needle was introduced into the cord root. However, before it was possible to aspirate a specimen of fetal blood for analysis, the needle became dislodged. Active bleeding from the cord root into the amniotic cavity was noted with subsequent formation of several large floating clots. Attempts to reenter the vessel were unsuccessful and were associated with episodes of bradycardia from 60 to 100 bpm. All further attempts to enter the cord were then abandoned and an emergency cesarean section was performed. The newborn infant weighed 1445 gm and had a hematocrit of 11%. A small fresh hematoma was present over the umbilical vein at the cord root. The infant required a total of seven exchange transfusions during the first 5 days of life and was subsequently discharged in good condition.

Comment

Locating the transfusion site. Accurate location and adequate visualization of the cord root is mandatory to successfully perform a transfusion in utero. In the absence of oligohydramnios it is extremely difficult to pierce the wall of a vessel in a mobile portion of the cord because it rolls or floats away from the needle tip. However, within 1 to 2 cm of the cord's insertion into either the placenta or fetal abdominal wall, movement of the cord is restricted and needle entry is therefore facilitated. The insertion of the cord into the placenta is the preferred site because fetal movement is more likely to interfere with venipuncture at the umbilicus.

Placentas in an anterior position are easier to work with than those that are posterior because one can usually approach the cord insertion through the placenta without having to enter the amniotic cavity. This, of course, minimizes the opportunity for the fetus to physically interfere with the seating of the needle tip. The cord insertion site is usually located easily in these cases unless the fetal body presses tightly against it. In these cases either manipulation of the fetus or lying the patient on her side will usually permit better visualization of the target site. If the cord is hanging straight down into the amniotic fluid the sides of the vessels may be difficult to see with clarity. This problem is overcome by using high-resolution scanners and, when in doubt, magnified images. It should be noted that the cord root will always be located in close proximity to large chorionic-plate vessels. If these vessels are not seen in adjacent scanning cuts the suspected cord root is probably an artifact.

When the cord inserts into a lateral fold of the placenta, venipuncture may be difficult if the needle approaches the vessel at right angles. In that situation the cord tends to be pushed into the posterior aspect of the placental substance until it disappears from view. It is often easier in these cases to roll the patient on her side so that access can be achieved to the base of the cord as it falls away from the placenta.

When the cord inserts into a posterior placenta, the cord root may be completely obscured by the fetal body lying over it. In this case the fetus must be manipulated and the mother rotated so as to locate the insertion site. In addition to having the problem of gaining access to the cord root, the distance from the maternal abdominal wall will be significantly greater than that for placentas in the anterior position. This invariably means that fairly long needles will be required for the procedure.

In some cases a central cord insertion in a placenta on the posterior uterine wall may be virtually impossible to access because of inability to move the fetus away from it. In those cases the operator might choose to perform an intravascular transfusion into the cord at its entry site into the abdomen or into the umbilical vein within the liver. A third possibility is to perform an intraperitoneal transfusion, which is a particularly good alternative in a fetus without significant hydrops. We recently had such a case. An intravascular transfusion at 25 weeks had been uneventful, but the cord root was found to be completely inaccessible at 27 weeks and again at 30 weeks. We chose to perform an intraperitoneal transfusion on the latter two occasions without trying an intravenous transfusion and both procedures were accomplished easily.

Premedication. The purpose of medicating patients before performing invasive intrauterine procedures is to minimize the patient's discomfort, reduce fetal and uterine activity, and prevent the introduction of infection. A standard regimen we have used during the past several years to accomplish these objectives in patients with no allergic contraindications is a combination of meperidine (50 to 100 mg), prochlorperazine (10 mg), diazepam (5 to 10 mg), and cefazolin (1 gm) administered intravenously approximately 15 minutes before beginning the procedure. One problem encountered with this drug combination is that the patient may fall asleep and breathe quite deeply, which results in considerable abdominal wall movement during the procedure. When this happens she can easily be awakened and told to think about breathing in a more normal fashion. It is often necessary to repeat this several times during the procedure and it is wise to warn the patient about it before administering the medication.

Another problem that may occur is the development of uterine contractions after the needle has been introduced. In the second case described in this report a contraction literally lifted the needle tip out of a cord vessel during one of the transfusions. As a result we now routinely administer ritodrine, 3 mg intravenously, along with the other premedications. This regimen was described by Caritis et al.[2] and we have found it to be a quick and effective way to provide tocolysis during the procedure.

Fetal movement with subsequent violent displacement of the needle tip has been a significant problem in some of our cases in which the amniotic cavity had been traversed to reach the cord root. In most of these cases the fetus was initially quite immobile but became increasingly more active as the transfusion progressed. This may have been because the sedatives given to the mother had initially only appeared to be effective, or that their effects were wearing off, or potentially that the transfused blood was having a dilutional effect and consequently lowering effective blood levels. Regardless of the explanation, sudden fetal movement can result in disruption of the procedure and possibly cause a life-threatening fetal hemorrhage by creating a large rent at the transfusion site. Ch de Crespigny et al.[3] have developed a technique of transiently paralyzing the fetus to overcome the problems associated with fetal movement during the procedure. These authors describe injecting 3 mg/kg (based on estimated weight) *d*-tubocurarine intramuscularly into the upper outer aspect of the fetal thigh approximately 30 minutes before the procedure is begun. They report using this technique on 12 occasions with complete paralysis being produced in 11 cases and partial, but adequate, curarization achieved in the remaining fetus. In all cases resumption of fetal movement was perceived by the mother 3 to 5 hours after the drug was administered and no adverse effects of the medication or its injection into the thigh have been detected. One fetus in that series was paralyzed on four separate occasions before undergoing intrauterine transfusions.

It should be noted that Ch de Crespigny et al.[4] perform intravascular transfusions into the umbilical vein within the fetal liver. With this approach it is obviously beneficial to immobilize the fetus as completely as possible before every transfusion to maximize the potential of successfully entering a vessel within the abdominal cavity. However, if transfusion is performed into a cord vessel, complete immobilization of the fetus is often not necessary. This is particularly true of cases in which the cord root is entered through the placental substance without traversing a portion of the amniotic sac. On the other hand, the first and fourth cases in this report were technical failures resulting in the performance of emergency cesarean sections precisely because fetal movement physically displaced the needle tip from a cord vessel that had been entered from the amniotic cavity.

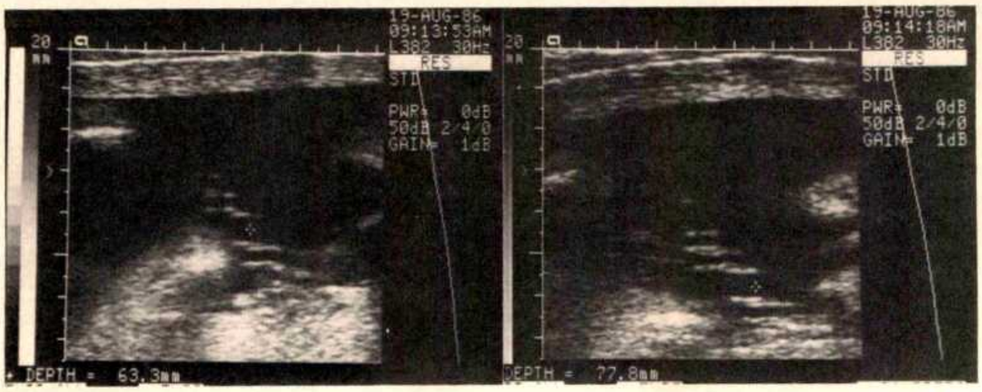

Fig. 1. The marker (+) is within the lumen of the umbilical vein at the cord insertion into a placenta in the posterior position. When pressure caused by the linear-array transducer is reduced, the measured distance from the maternal abdominal wall increases from 63.3 mm *(left)* to 77.8 mm *(right)*.

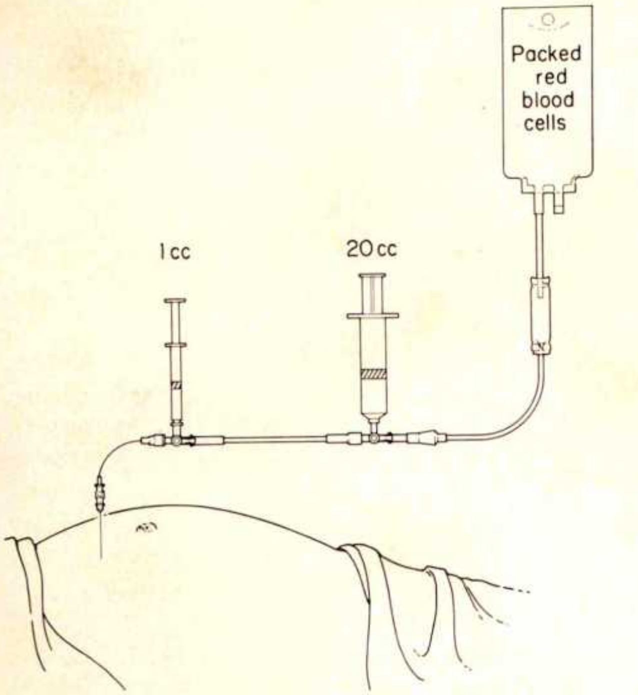

Fig. 2. This setup for transfusing intravascularly is designed to minimize movement at the needle hub. A series of two three-way stopcocks permits aspiration of samples for analysis and transfusion of measured amounts of blood without disconnecting the tubing from the needle during the procedure.

We have not yet paralyzed any of the fetuses transfused at Mt. Sinai but feel that some of our failures might have been avoided if we had done so. It is clear that paralysis is not necessary in most cases, but when the cord root must be entered through the amniotic cavity, direct fetal neuromuscular blockade may be extremely valuable. When fetal paralysis is felt to be desirable the intravascular use of a fairly short-acting agent such as atracurium besylate may have significant theoretic advantages over the intramuscular administration of tubocurarine.

Choosing a needle. We have used needles with a central stylet and conventional bevel for all of our transfusions. The length of the needle to be used is obviously determined by the distance from the maternal abdominal wall at the chosen entry site to the lumen of the target vessel. This can be measured directly from the ultrasound display, but care must be taken not to artifactually shorten the distance by putting too much pressure on the transducer. The resultant reduction in distance may be significant (Fig. 1). We have also found that it is wise to use a needle that is at least 2 cm longer than the measured distance because of underestimation of the required needle length. This discrepancy may be due to movement of the vessel away from the needle tip, minimal contractions, or scanning artifacts.

Although small-gauge needles are desirable to minimize trauma to vessel walls, these may be too flexible to allow optimal control of the needle tip at depths of 9 to 15 cm from the maternal abdominal wall. Furthermore, the transfusion of tightly packed cells through small-gauge needles is slow, may cause hemolysis, and predisposes to small clot formation because of the restricted rate of infusion. Therefore, despite the use of smaller gauge needles advocated by other groups, we use 22-gauge needles for blood sampling and transfusions up to 24 weeks and 20-gauge needles for transfusions at later gestational ages.

Analysis of the aspirate. After the needle appears to be properly seated within the target vessel on the ultrasound display, the central stylet is removed and a sample of blood is aspirated into a heparinized 1 ml syringe for analysis. Although only a fraction of a milliliter is necessary for cell sizing, it is advisable to withdraw a full milliliter to avoid dilutional effects of the heparin. Cell sizing and hematocrit determinations should be done in a facility that is immediately adjacent to the procedure room to diminish transport time and time involved in reporting results of the analysis.

Cell sizing is performed to be certain that the aspirated blood is fetal when compared with a sample of the mother's blood drawn before the procedure is begun. This method of distinguishing between the samples is dependent on the fact that the mean corpuscular volume of fetal red blood cells is significantly higher than that of adult red blood cells. However, this analysis becomes less useful after intrauterine transfusions have been initiated because the majority of red cells in the fetal circulation will often be comprised of previously transfused adult cells. In these situations the low hematocrit values and yellow serum of the aspirated fetal blood will usually differentiate it from that of the mother. Furthermore, it can be verified that the fetal vasculature has been entered by viewing flow within the cord or chorionic plate vessels on the ultrasound display as the transfused blood is injected.

If blood is aspirated directly from the needle the syringe must be detached from the needle's hub and the transfusion tubing inserted in its place after the initial sample has been withdrawn. The tubing for the transfusion is attached to a three-way stopcock, which in turn attaches to a 20 ml syringe and the bag of packed cells to be transfused. This standard setup allows blood to be loaded into the syringe for administration at a controlled rate without disconnecting the system. However, if the tubing from the three-way stopcock leads directly to the needle hub, the system must be "broken" whenever a sample of blood is aspirated to check the hematocrit. Each time this is done movement of the hub may inadvertently cause displacement of the tip. In the third case described above this occurred and was responsible for our inability to perform the transfusion. The problem could be avoided by placing a short length of flexible tubing into the needle hub leading to a three-way stopcock to which an aspirating (e.g., 1 ml) syringe is attached. The transfusion tubing would then be attached to the third portal (Fig. 2). By using a setup of two three-way stopcocks in this fashion it should not be necessary to "break" the system at all until the transfusion is completed.

When blood is withdrawn for analysis, the first 0.5 to 1 ml aspirated should be discarded if the transfusion has been started to remove any of the packed cells left in the needle or attached tubing. The hematocrit value can be determined by spinning samples of aspirated whole blood in standard microcapillary tubes. However, this takes 2 to 3 minutes, which can seem like a very long time when one is waiting for the result with a needle placed inside a fetal vessel. Computer attachments to some cell-sizer models allow rapid hematocrit and mean corpuscular volume determinations to be performed in seconds. This obviously can help to shorten the procedure time but prevents the hyperbilirubinemia visible on most initial spun hematocrits from being used as a marker for fetal blood.

In conclusion, we have found intravascular blood sampling and transfusion to be of great usefulness in the rational management of erythroblastosis fetalis. Technical problems associated with these procedures can be reduced by using needles of appropriate design, length, and gauge, selecting the insertion site with care, premedicating the mother and her fetus wisely, and using a delivery system that minimizes the need to move the needle hub. A rationale for intravenous muscle blockade of the fetus in selected cases is presented, although, to our knowledge, this has not yet been attempted. Finally, we would like to restate that unless severe hydrops is present, an intraperitoneal transfusion should always be strongly considered as an alternative to performing an intravascular transfusion that seems as if it will be unusually difficult to perform.

REFERENCES

1. Berkowitz RL, Chitkara U, Goldberg JD, et al. Intrauterine intravascular transfusions for severe red blood cell isoimmunization: ultrasound-guided percutaneous approach. AM J OBSTET GYNECOL 1986;155:574.
2. Caritis SN, Lin LS, Wong LK. Evaluation of the pharmacodynamics and pharmacokinetics of ritodrine when administered as a loading dose. AM J OBSTET GYNECOL 1985;152:1026.
3. Ch de Crespigny L, Robinson HP, Ross AW, Quinn M. Curarisation of fetus for intrauterine procedures. Lancet 1985;i:1164.
4. Ch de Crespigny L, Robinson HP, Quinn M, et al. Ultrasound-guided fetal blood transfusion for severe rhesus isoimmunization. Obstet Gynecol 1985;66:529.

Isolation of acquired immunodeficiency syndrome virus from the placenta

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The etiologic agent of the acquired immunodeficiency syndrome is human immunodeficiency virus. We report here a case of the culturing of this agent from the placenta and suggest the clinical implications. (AM J OBSTET GYNECOL 1987;157:10-1.)

Key words: Acquired immunodeficiency syndrome, placenta, isolation, human immunodeficiency virus

There is little published information on the culturing of human immunodeficiency virus (HIV), the etiologic agent of the acquired immunodeficiency syndrome (AIDS), from mothers and infants at risk for the disease. The virus has been cultured from breast milk and it has been suggested that AIDS in neonates may result from breast-feeding.[1] HIV has been cultured from the lymphocytes of mothers at risk for the development of AIDS. Transplacental transmission of the HIV virus has also been reported. We present here a report of the isolation of the HIV at the time of delivery from the placenta of a patient at risk for the development of AIDS.

Case presentation

The patient was a 24-year-old, white woman, gravida 2, para 1, whose prenatal course was complicated by sexual exposure to a partner who had AIDS as a result of intravenous drug abuse. At 10 weeks' gestational age the patient had positive enzyme-linked immunosorbent assay, Western blot, and immunofluorescence assay serologic test results for the anti-HIV antibody. Although asymptomatic, a positive peripheral blood lymphocytic culture for the HIV virus was also yielded in this woman at 26 weeks' gestational age. Special T-cell studies at that time showed a reversed T4/T8 ratio. The patient's pregnancy was otherwise uncomplicated. Sonographic examinations of the fetus showed no evidence of intrauterine growth retardation. The patient had an uncomplicated labor and was delivered at 39 weeks' gestational age of a 3700 gm male infant. There were no neonatal complications. The umbilical cord blood had positive enzyme-linked immunosorbent assay, Western blot, and immunofluorescence assay serologic test results for the anti-HIV antibody. The infant has not developed symptoms of

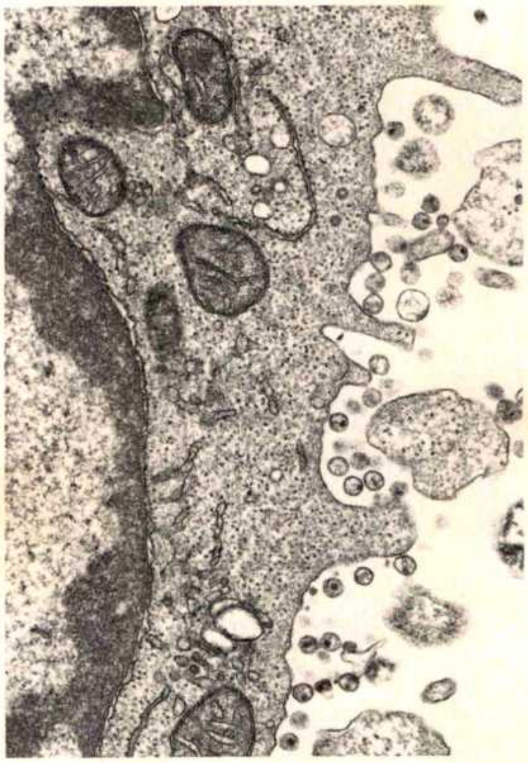

Fig. 1. Portion of a human T cell with extracellular HIV (Original magnification ×35,000.) (Photograph courtesy of Robert J. Munn, Department of Medical Pathology, School of Medicine, University of California, Davis.)

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AIDS. Viral cultures of the cord blood, lochia, and baby were negative; however, cultures of the placenta were positive for HIV (Fig. 1).

Comment

Neonatal AIDS can result from contact of the baby with maternal blood during delivery. There is presently no evidence of this occurring in this newborn infant. The positive viral status of this patient was detected in our screening of pregnant women at risk for AIDS. The positive viral culture of the placenta was probably a result of the patient's positive blood culture. HIV-positive pregnant patients have a risk of passing this infection to the neonate. Transmission may occur through breast milk, through transplacental transfer, or by contact with maternal blood during delivery. The extra risk of this transfer remains unclear. The placenta must be handled carefully by health care workers and housekeeping personnel to decrease exposure to the virus. Recommendations for the prevention of perinatal transmission of HIV and AIDS have been previously published and should be reviewed by individuals providing obstetric care.[2]

REFERENCES

1. Ziegler JB, Johnson RO, Cooper DA, Gold J. Postnatal transmission of AIDS-associated retrovirus from mother to infant. Lancet 1985;2:896-8.
2. Centers for Disease Control. Recommendations for assisting in the prevention of perinatal transmission of human T-lymphotropic virus type III/lymphadenopathy-associated virus and acquired immunodeficiency syndrome. MMWR 1985;34:721-32.

Management of preterm premature rupture of membranes: Lack of a national consensus

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A questionnaire concerning the management of women with preterm premature rupture of membranes was sent to the members of the Society of Perinatal Obstetricians. Expectant management was recommended by 97% of the respondents. There was no consensus about the role and frequency of steroids, blood work, ultrasound examination, and fetal monitoring in the follow-up of these patients. (AM J OBSTET GYNECOL 1987;157:11-2.)

Key words: Preterm rupture of membranes, steroids, management

Optimal management for patients with premature rupture of the membranes before term remains controversial. A recent review[1] cites conflicting results from numerous studies that have attempted to determine the benefit of various treatment modalities. We attempted to determine if there was a consensus among perinatologists regarding the use of steroids in patients with preterm premature rupture of membranes.

Material and methods

A questionnaire was sent in May 1985 to members of the Society of Perinatal Obstetricians asking the following questions: (1) Do you use steroids between 28 and 32 weeks in patients with premature rupture of the membranes? If yes, which steroid and what dose are used? (2) Do you recommend induction after a course of steroids? (3) Do you recommend no intervention after premature rupture of the membranes? (4) Do you repeat steroid therapy after 7 to 10 days in a patient with premature rupture of the membranes who has not delivered? (5) Do you use intravenous and/or oral tocolytics if a patient begins labor during steroid use? (6) Conservative management means: (A) Bed rest in the hospital? (B) Bed rest either in the hospital or home? (7) What laboratory work do you use to monitor a patient with premature rupture of the membranes? (8) How frequently do you perform ultrasonography?

Results

A total of 492 questionnaires were mailed, and 285 were returned, which represented a 58% response rate. Responses were received from 167 hospitals in 114 cit-

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Table I. Comparison of expectant management protocols between steroid users and nonsteroid users

	Steroids (N = 98)		*No steroids (N = 163)*		*All respondents (N = 261)*	
Tocolytic therapy	83	85%	11	7%	94	36%
Conservative management; hospital or home	29	30%	60	37%	89	34%
White blood count	83	85%	99	61%	182	70%
Vital signs	12	12%	45	28%	57	22%
Fetal monitoring	26	26%	37	23%	63	24%
C-reactive protein	17	16%	27	16%	44	17%
Cervical cultures	25	25%	36	22%	61	23%
Vaginal phosphatidylglycerol	9	9%	16	10%	25	10%
Amniocentesis	29	30%	36	22%	65	25%
Ultrasound examination	92	94%	153	94%	245	94%

ies in the United States and Canada. Forty-two percent of obstetric residency programs and 90% of maternal-fetal fellowship programs were represented.

Steroid administration was routinely recommended by 39% (113), occasionally or rarely recommended by 4% (nine), and never recommended by 57% (163) of the respondents. Of respondents representing fellowship programs, 40% recommended steroid administration. The lack of agreement on this issue is reaffirmed by the fact that of 47 fellowship programs with more than one respondent to the questionnaire, 17 (36%) had disagreement within the faculty as to the benefit of steroid administration.

Of the physicians who routinely administered steroids, 6% (seven) recommended an induction after steroid therapy, and 94% (106) recommended expectant management. Betamethasone, 12 mg, was prescribed by 91% (102), but the time course was variable. The most popular regimen, two doses 24 hours apart, was given by 57% (53), but 26% (25) used two doses 12 hours apart. Only nine physicians used dexamethasone, and only two agreed on the dose and time of administration. Of the 113 who routinely used steroids for preterm premature rupture of the membranes, 98 detailed further management plans. Readministration of steroids to patients who remained undelivered was advocated by 53% (51).

A comparison of the management schemes between the two groups (steroid users and nonsteroid users) is shown in Table I. Tocolytic therapy was much more frequent in the group who used steroids. Only one third of each group felt that a patient could be managed conservatively at home. The percentage of physicians who stated that they used the tests are listed in Table I. Within each group, there was great variability in the frequency in which these tests were ordered. For example, only 36% of the respondents used daily fetal monitoring; an additional 27% monitored every 3 to 4 days. More than 94% of both groups used at least an initial ultrasound examination on admission, and 54% to 57% continued with ultrasound examinations on at least a weekly basis. Fluid assessment and biophysical profiles were the most frequent reason given for repeated ultrasound examination.

Comment

The results of this survey demonstrate a lack of concensus regarding the optimal management of preterm premature rupture of the membranes. This conclusion was reached with considerable confidence, since responses were obtained from 90% of maternal-fetal fellowship programs, the very institutions in which protocols are designed and high-risk care is delivered. Although there is an overwhelming concensus regarding the value of expectant management, the respondents are divided on the benefit of steroid administration in this high-risk group. Physicians who give steroids are more likely to give tocolytic therapy, use frequent white blood counts, and keep their patients in the hospital. However, there is a great diversity of opinion regarding the role and frequency of blood work, fetal monitoring, and prolonged hospitilization in the follow-up of these patients. Documentation of a lack of consensus, as provided by this survey, is important because of obvious implications in the areas of standards of patient care, medical and hospital costs, design of future studies, and medical liability.

REFERENCE

1. Mead PB. Management of the patient with premature rupture of membranes. Clin Perinatol 1980;7:243.

Reduction of group B streptococcal maternal and neonatal infections in preterm pregnancies with premature rupture of membranes through a rapid identification test

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To establish the effect of rapid detection and chemoprophylaxis of group B streptococcal genital colonization in preterm gestations with premature rupture of membranes, 260 singleton pregnancies were cultured. Through the use of a rapid test based on coagulation methods, group B streptococcus was identified in 84 (32%) patients, of which 18 (7%) had heavily colonized infections. The treatment of 36 patients resulted in no cases of chorioamnionitis or neonatal sepsis compared with 11 (23%) cases of chorioamnionitis and 13 (27%) of neonatal sepsis among 48 untreated patients ($p < 0.01$). Although the risk of infection was significantly higher in patients with heavily colonized infections, characterized by <5 hours' growth for detection, even light colonization, requiring 20 hours for detection, resulted in a 14% rate of maternal and 16% rate of neonatal infection, respectively. (AM J OBSTET GYNECOL 1987;157:13-6.)

Key words: Maternal and neonatal infections, premature rupture of membranes, group B streptococcus, rapid identification test

Group B streptococcus is the leading cause of neonatal infection, with an incidence in the term population between one and four per 1000 live births.[1,2] However, the attack rate is relatively low considering that approximately 20% of pregnant women are symptom-free carriers. Several studies[3,4] have demonstrated the effectiveness of intravenously administered ampicillin during the intrapartum period in significantly reducing group B streptococcus disease in the term neonate. However, this approach requires serial antenatal genital cultures to identify the 20% who are group B streptococcus carriers and then exposure of this 20% to ampicillin to prevent neonatal sepsis in 0.7% of these patients. In a recent study[5] a rapid test based on coagglutination methods identified within 5 hours a subset of patients at high risk of delivering infants destined to develop group B streptococcus. This high-risk group representing 6% of the obstetric population was characterized by a heavy group B streptococcus genital colonization (colony counts $>3 \times 10^4$/swab). The intrapartum treatment of 37 term patients with heavily colonized infections resulted in no cases of neonatal group B streptococcus sepsis compared with 10% in an untreated group. On the other hand, no case of group B streptococcus was recorded in a group of 98 untreated term patients with lightly colonized infections.

Conservative management of preterm patients with premature rupture of membranes has been accepted by most clinical investigators in an attempt to reduce the serious neonatal complications of prematurity, mainly respiratory distress syndrome and intraventricular hemorrhage. Nevertheless, the risk of clinical maternal intra-amniotic infection ranges from 24% in pregnancies <28 weeks to 5% in those 33 to 34 weeks.[6] Infections not only translate into serious maternal morbidity but also compound the problem of prematurity in the infants. Although no direct cause-effect relationship has been identified between group B streptococcal genital colonization and premature rupture of membranes, a number of studies[7,8] have reported a higher incidence of group B streptococcal colonization in pregnancies complicated by preterm births. Group B streptococcus has been identified as the leading cause of chorioamnionitis. Thus not only is the unborn preterm fetus at risk of group B streptococcus infection after premature rupture of membranes, but the risk is even greater with maternal group B streptococcal intra-amniotic infections. Further at issue is the beneficial use of antenatal corticosteroids in preterm patients with premature rupture of membranes. Although some investigators have reported a reduction in respiratory distress syndrome after corticosteroid administration,[9,10] some[11,12] have found this benefit offset by an increased risk of maternal and neonatal infection.

The purpose of this study is to establish whether the rapid identification and treatment of carriers of

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Table I. Colonization rates and magnitudes in preterm and term patients

	Preterm (n[%])	*Term (n[%])*	*p Value*
Number	260	1207	
Group B streptococcal positive colonized	84 (32)	263 (22)	0.05
Heavily colonized	18 (7)	67 (5.5)	NS
Lightly colonized	66 (23)	196 (16)	NS

group B streptococcus would result in reduced maternal and neonatal infection in the conservatively managed preterm patient with prematurely ruptured membranes.

Material and methods

Between June 1984 and June 1986, 260 patients were studied at Orlando Regional Medical Center with a single intrauterine pregnancy ≤ 34 weeks and premature rupture of membranes >12 hours before onset of labor. Rupture of membranes was documented by obvious pooling with sterile speculum and confirmed by ferning and alkaline pH by Nitrazine paper. Gestational age was established by menstrual history, initial physical examination, and previous ultrasound report. On admission to the hospital each patient underwent an ultrasound evaluation to confirm gestational age, placental location, fetal position, and oligohydramnios. A reactive nonstress test was required for gestations >28 weeks. If the test was nonreactive after 40 minutes, it was repeated within 24 hours after breakfast, and if the nonreactivity persisted, ultrasonography was performed to verify fetal breathing movements and truncal movements in a 30-minute evaluation. Excluded from the study were all patients in labor at the time of admission to the hospital, those with foul-smelling fluid, ultrasonographic evidence of intrauterine growth retardation or nonreassuring antenatal test, those with phosphatidylglycerol in the amniotic fluid obtained from vaginal pooling, and neonates with congenital anomalies incompatible with life. Whenever possible, patients received 24 mg dexamethasone in four divided intramuscular doses 12 hours apart. In the absence of infection, patients in labor were treated with magnesium sulfate to achieve completion of steroid treatment. When magnesium sulfate failed to arrest labor after 1 hour at maximum dosage of 4 gm/hr or a serum level of 9 mg/dl, ritodrine hydrochloride was added to a maximum dose of 0.35 mg/min.

On admission to the hospital, in addition to aerobic vaginal cultures, a rapid screen test for group B streptococci based on coagglutination methods was performed from a vaginal swab. As described previously, a positive identification at 5 hours is associated with heavy colonization whereas a positive result at 20 hours implies light colonization. After a report of positive identification of group B streptococcus, the patient was treated intravenously with 1 gm ampicillin every 6 hours, and expectant management was continued in the absence of clinical evidence of intra-amniotic infection. If the patient was allergic to ampicillin, a first-generation cephalosporin was used. The rapid-screening test was repeated in 48 hours to confirm the eradication of the organism, and after this report the patient was given 500 mg oral ampicillin every 6 hours until labor began. In the intrapartum period the patients were again treated with 1 gm intravenous ampicillin until delivery.

After delivery the infant was examined by one of four attending neonatologists. Surface, oropharynx, urine, blood, and cerebrospinal fluid cultures were obtained on admission to the neonatal intensive care unit before antibiotic therapy was started. A urine Wellcogen latex agglutination test was performed on all neonates for detection of group B streptococcal antigen.[13] The diagnosis of neonatal sepsis was made on the basis of a positive blood or cerebrospinal fluid culture or positive Wellcogen urine screen and oropharynx cultures along with radiographic and clinical signs of congenital infection as determined by the attending neonatologist. Respiratory distress syndrome was diagnosed by the attending neonatologist on the basis of characteristic radiologic findings along with increasing ventilatory requirements.

Analysis of the data was performed by means of χ^2 and two-tailed t tests. Differences were considered significant at a probability level of 0.05.

Results

Two hundred sixty patients with preterm pregnancies with premature rupture of membranes qualified for the study, and of these, 84 (32%) were found to have colonies of group B streptococci, 18 of which (7%) had heavy colonization. During the same period of study, 1207 term patients were screened with the same rapid test, and of these 22% tested positive for group B streptococci, statistically significantly different from the preterm population with premature rupture of membranes ($p < 0.05$), as shown in Table I. Thirty-six (43%) of the preterm patients received antibiotic therapy in accordance with the protocol outlined in the previous section, and none of these infants developed group B streptococcal sepsis despite the concurrent use of dexamethasone and expectant management. Primarily because of a short latency period or delay in processing the screens, 48 patients did not receive antibiotic therapy and 11 (23%) developed chorioamnionitis. In addition, 17 (35%) of the infants from the untreated group of 48 mothers had a positive urine Wellcogen screen, statistically higher than the incidence

Table II. Effect of ampicillin treatment

	Maternal colonization			
	Heavy (N = 18)		*Light (N = 66)*	
	T	*U*	*T*	*U*
Number	7	11	29	37
Chorioamnionitis (No.[%])	—	* 6 (55)	—	† 5 (13.5)
Neonatal sepsis (No.[%])	—	* 7 (64)	—	† 6 (16)
Group B streptococcal antigen–positive urine screen (No.[%])	1 (14)	* 8 (73)	1 (3)	† 9 (24)

T = Treated; U = untreated.
$^*p < 0.05$.
$^\dagger p < 0.01$.

in the treated population (2/36; 5.5%; $p < 0.01$). Of these 17 infants with positive Wellcogen screens, 13 (27%) developed neonatal sepsis. The data summarized in Table II indicate that indeed the incidences of chorioamnionitis, positive neonatal urine Wellcogen screen, and sepsis were significantly higher in those patients with heavy colonization. Table III summarizes the data on the 48 untreated subjects. The 37 mothers who did not develop chorioamnionitis were of more advanced gestational age than those who did (31.8 versus 28.1 weeks). Of the 11 patients with chorioamnionitis, those with heavy colonization (i.e., screen positive at 5 hours) were of more mature gestations (29.5 versus 26.2 weeks) than were those with chorioamnionitis and light colonization. The patients with heavy colonization developed signs of chorioamnionitis sooner after rupture of membranes than did those with light colonization (1.86 versus 2.45 days).

Table III. Effect of gestational age and level of colonization on chorioamnionitis in untreated subjects

Chorioamnionitis	*Colonization*	*Gestational age (wk)*	*LAT period (days)*
Yes (N = 11)	Heavy (N = 6)	29.5	1.96
	Light (N = 5)	26.2	2.45
No (N = 37)	Heavy (N = 5)	32.8	3.19
	Light (N = 32)	31.3	4.08

Comment

A previous study demonstrated that through the use of a rapid-screen test based on coagglutination methods, the population destined to deliver term infants at high risk to develop group B streptococcal sepsis could be identified within 5 hours as those patients with heavy genital colonization. In that study their intrapartum treatment with intravenous ampicillin essentially prevented the vertical transmission to their infants. The study also showed that term infants born to mothers with light colonizations of group B streptococci do not appear to be at risk to develop sepsis.

The purpose of the present study was to determine the effectiveness of this rapid test to screen preterm patients with premature rupture of membranes managed conservatively with the concurrent use of dexamethasone and to establish the safety of treating the patients with colonization while still continuing an expectant management of the pregnancy. The study demonstrated that the rapid identification and treatment of the symptom-free patient with colonization resulted in no cases of maternal or neonatal infection. Failure to treat the colonized preterm patient with premature rupture of membranes resulted in a high incidence of chorioamnionitis (25%) and neonatal sepsis (35%). The risks were higher in those with heavy colonization. Moreover, as summarized in Table IV, contrary to the experience in the term population, the untreated preterm subject with light colonization is at significant risk of chorioamnionitis (13%) and neonatal sepsis (16%), the risks inversely related to gestational age. This inverse relationship suggests an ability of amniotic fluid to more successfully inhibit group B streptococcal infections with advancing gestational age. This inhibitory capacity of amniotic fluid is further illustrated by the data on chorioamnionitis in Table III. Note that not only were the pregnancies of patients who developed intra-amniotic infection with light colonization more immature, but their latency periods were longer and, presumably because of this longer latency period, more and more of the protective capacity of amniotic fluid was lost with continued loss of fluid volume. This continued decrease in fluid volume was documented ultrasonographically.

The data from the study indicated a 32% rate of group B streptococcal colonization in the group of preterm patients with rupture of membranes. This is significantly higher ($p < 0.05$) than the colonization rate

Table IV. Effect of gestational age and colonization magnitudes in maternal and neonatal infections of untreated subjects

	Maternal colonization					
	Heavy			*Light*		
	T		*P*	*T*		*P*
Number	30		11	98		37
Chorioamnionitis (No.[%])	5 (17)	*	6 (5)	1 (1)	†	5 (13.5)
Neonatal sepsis (No.[%])	3 (10)	*	7 (64)	—	†	6 (16)
Group B streptococcal antigen–positive urine screen	8 (27)	*	8 (73)	1 (1)	†	9 (24)

T = Term; P = preterm.
*$p < 0.05$.
†$p < 0.01$.

of 22% in a group of term patients with intact membranes and suggests an association between genital colonization with the organism and premature rupture of membranes. Moreover, six of the 11 (55%) patients with chorioamnionitis were clinically diagnosed within 48 hours after rupture of the membranes. Hence for all mothers to receive the benefits of antenatal antibiotic therapy requires the patient to be admitted to the hospital soon after rupture of the membranes. The screen must be performed rapidly and be able to be processed around the clock with efficient reporting of results to the obstetrician. Therefore, until a direct screening test for group B streptococci is available, a strong argument could be made for the antenatal use of ampicillin in preterm patients with premature rupture of membranes until the results of the rapid screen became available. This approach would result in, at most, three doses of intravenous ampicillin to the group of patients not colonized with group B streptococci. This approach is not expected to result in the selection of resistant organisms.

REFERENCES

1. Baker CJ. Group B streptococcal infections in neonates. Pediatr Rev 1979;1:5.
2. Pass MA, Gray BM, Kate S, et al. Prospective studies of group B streptococcal infections in infants. J Pediatr 1979;95:437.
3. Yow MD, Mason ED, Leeds LJ, et al. Ampicillin presents intrapartum transmission of group B streptococcus. JAMA 1979;241:1245.
4. Boyer KM, Gadzala CA, Burd LI, et al. Selective intrapartum chemoprophylaxis of neonatal group B streptococcal early-onset disease. III. Interruption of mother-to-infant transmission. J Infect Dis 1983;148:810.
5. Morales WJ, Lim DV, Walsh AF. Prevention of neonatal group B streptococcus sepsis by the use of a rapid screening test and selective intrapartum chemoprophylaxis. AM J OBSTET GYNECOL 1986;155:979.
6. Morales WJ, Lazar AJ, Washington SR. The effect of chorioamnionitis on perinatal outcome in preterm gestation. J Calif Perinatol Assoc 1984;2:69-70.
7. Regan JA, Chao S, James LS. Premature rupture of membranes, preterm delivery, and group B streptococcal colonization of mothers. AM J OBSTET GYNECOL 1981; 141:184.
8. Moller M, Borch K, Thomsen AC, et al. Rupture of membranes and premature delivery associated with group B streptococci in urine of pregnant women. Lancet 1984; ?:69.
9. Morales WJ, Diebel ND, Lazar AJ, et al. The effect of antenatal dexamethasone administration on the prevention of respiratory distress syndrome in preterm gestations with rupture of membranes. AM J OBSTET GYNECOL 1986;154:591.
10. Mead PB, Clapp JF. The use of betamethasone and timed delivery in management of premature rupture of the membranes in preterm pregnancy. J Reprod Med 1977; 19:3.
11. Garite TJ, Freeman RK, Linzey EM, et al. Prospective randomized study of corticosteroids in the management of premature rupture of membranes and the premature gestation. AM J OBSTET GYNECOL 1981;141:508.
12. Simpson GF, Harbat GM. Use of betamethasone in management of preterm gestation with premature rupture of membranes. Obstet Gynecol 1985;66:168.
13. Ingram DL, Suggs DM, Pearson AW. Detection of group B streptococcal antigen in early-onset and late-onset group B streptococcal disease with the Wellcogen strep B latex agglutination test. J Clin Microbiol 1982; 16:656.

Obstetric outcome of patients with a previous episode of spurious labor

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The obstetric performance of patients admitted with spurious labor was evaluated. The incidence of fetal distress in labor was significantly higher in those who had spurious labor (16.7%) than in those who were established in labor within 24 hours of admission (3.8%). An equivocal or ominous fetal heart rate trace on admission was related to the occurrence of fetal distress in labor in both the spurious labor group and the normal labor group. Positive predictive value of a normal test was low in the spurious labor group. Obstetric interventions such as augmentation (35.7%) and operative deliveries (41.0%) were significantly higher in the spurious labor group than in the normal labor group (19.7% and 18.8%, respectively). The induction rate in the spurious labor group was 15.5%. The use of oxytocin for augmentation and induction of labor seems to contribute to the increased incidence of fetal distress and operative deliveries in the spurious labor group. The spurious labor group also had a higher incidence of fetal distress than the control group even among the patients who did not receive oxytocin. The results suggest that patients with an episode of spurious labor constitute a high-risk group with a considerable risk of fetal distress and obstetric interventions in subsequent labor. (AM J OBSTET GYNECOL 1987;157:17-20.)

Key words: Obstetric outcome, fetal distress, augmented labor, spurious labor

Patients in spurious labor with cessation of labor after a period of painful uterine contractions form an interesting group and are a common problem in obstetric practice. The fact that the definite physiologic mechanism of the onset of spontaneous labor remains elusive makes it difficult to explain the occurrence of spurious labor.

Because of the lack of knowledge about the cause of spurious labor there is no uniform policy in management of these cases at term. Some clinicians may apply a policy of routine induction of labor while others take a more conservative attitude and discharge the patients after a period of observation after a reassuring fetal heart rate trace has been obtained. There is also an overall uncertainty as to how to settle the diagnosis. Friedman[1] suggested a therapeutic test with sedation or analgesic used to differentiate false labor from those who are in a protracted latent phase of labor.

There is little knowledge in the literature about the obstetric outcome of these patients and opinions are conflicting. Quinn et al.[2] reported no adverse outcome whereas Beazley and Lobb[3] expressed concern about these patients and felt that they deserved special attention. More research is needed in this area; thus we were prompted to perform this investigation. We studied the obstetric performance in a group of patients with a previous period of spurious labor at term, that is, painful contractions recorded at least once in every 10 minutes on admission but no established labor within the next 24 hours. Spontaneous abatement of the contractions occurred without the use of sedatives or analgesics. The diagnosis of spurious labor was thus made retrospectively. The obstetric and neonatal outcomes of this group were compared with those of patients with established labor within 24 hours of admission (control group). The fetal and neonatal outcomes in these two groups were related to a 20-minute fetal heart rate trace on admission.

Patients and methods

Patients admitted to the Kandang Kerbau Hospital, Singapore, during the working hours of 8:30 AM to 3:00 PM each day except Sundays, during the period of February 1, 1984, to December 1, 1985, with painful uterine contractions, with or without show but without leaking liquor and high-risk antenatal factors, were considered for the study. Within minutes of entering the hospital, an admission test (a 20-minute fetal heart rate trace) was performed in all patients.[4] The trace was recorded in a concealed manner; the fetal pulse lamp was covered, the fetal heart rate was displayed digitally, and the volume of the two-channel chart recorders was turned off. An optimal trace was obtained by observing

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Table I. Incidence of fetal distress in labor in cases of previous spurious labor compared with control groups

	Patients with previous spurious labor (N = 168)		*Control group (N = 1041)*		
	n	%	*n*	%	*Significance*
Lower-segment cesarean section	16	9.5	18	1.7	$p < 0.001$
Forceps	10	6.0	16	1.5	$p < 0.001$
Normal delivery with low Apgar score needing admission to neonatal unit	2	1.2	6	0.6	NS
Total	28	16.7	40	3.8	$p < 0.001$

Table II. Fetal distress in the groups with previous spurious labor and control subjects in relation to outcome of admission test

	Patients with previous spurious labor				*Control group*			
	Reactive admission test		*Equivocal or ominous admission test*		*Reactive admission test*		*Equivocal or ominous admission test*	
	n	%	*n*	%	*n*	%	*n*	%
Incidence of fetal distress	22	14.4	6	37.5	31	3.2	9	15.3
	$p < 0.05$				$p < 0.001$			

the green indicator lamp suggesting good-quality signals in a Hewlett Packard* 8040 or 8041 cardiotocograph fetal monitor while placing the ultrasound transducer to detect the fetal heart rate.

The trace obtained was placed in a sealed envelope for later interpretation. The results of the admission test were categorized as reactive (normal), equivocal, or ominous. Criteria for reactive normal were: presence of two accelerations (>15 bpm, >15 seconds) within the test period, no accelerations but normal baseline rate and normal baseline variability (10 to 25 bpm), or early decelerations when accelerations were present. Criteria for equivocal were: normal baseline rate with no accelerations and baseline variability <5 bpm, abnormal baseline rate with reduced variability (5 to 10 bpm), or variable decelerations without ominous signs. Criteria for ominous were: abnormal baseline rate and baseline variability <5 bpm, repeated late decelerations, repeated variable decelerations with any of the ominous signs, duration <60 seconds, or beat loss >60 bpm, rebound tachycardia, slow recovery, and a late component.

Fetal distress was said to be present if an abdominal or vaginal operative delivery was performed for this indication (or in conjunction with another indication), judged by a cardiotocographic trace or fetal blood pH estimation, or if the baby was born spontaneously but with a low Apgar score and needing admission to the special care baby unit for this specific reason.

Patients diagnosed as being in spurious labor were sent to the antenatal ward and were discharged after 12 hours or more of observation; they were given an appointment to the antenatal clinic within 1 week. Those who were not established in labor after a 24-hour period but were within a week were considered for the study. Patients who were established in labor within 24 hours of admission in the first 6 months of our study formed the control group.

A computerized coding sheet was commenced at the time of admission with the details of age, parity, and antenatal complications, if any. Details of the incidence of fetal distress on electronic fetal monitoring, operative delivery, indication for operative delivery, neonatal outcome judged by 1- and 5-minute Apgar scores, birth weight, and admission to the special care baby unit were recorded on readmission of the patient to the labor ward and before the patient was discharged from the hospital. The obstetric outcome was correlated with the results of the admission test, and statistical significance was verified by use of Student's *t* test and χ^2 analysis.

The obstetric interventions of augmentation and induction of labor, emergency cesarean section, and instrument-assisted vaginal deliveries were evaluated in the spurious labor group and compared with those in

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*Hewlett Packard Ltd., Boeblingen, West Germany.

Table III. Rate of obstetric interventions in cases with previous spurious labor compared with incidence in normal labor group

	Patients with spurious labor (N = 168)		*Control group (N = 1041)*		
Obstetric intervention	*n*	*%*	*n*	*%*	*Significance*
Induction of labor	26	15.5	—	—	—
Augmentation of labor	60	35.7	205	19.7	p < 0.001
Emergency lower-segment cesarean section	34	20.2	83	8.0	p < 0.001
Operative vaginal delivery	35	20.8	112	10.8	p < 0.001

Table IV. Use of oxytocin and obstetric outcome

	Oxytocin used					*Oxytocin not used*				
	Spurious labor		*Control group*			*Spurious labor*		*Control group*		
	n	*%*	*n*	*%*	*Significance*	*n*	*%*	*n*	*%*	*Significance*
Total	86	51.2	205	19.7		82	48.8	836	80.3	
Incidence of fetal distress	22	25.6	18	8.9	p < 0.001	6	7.3	22	2.6	p < 0.05
Cesarean section rate	24	27.9	18	8.9	p < 0.001	10	12.2	65	7.8	NS
Instrument-assisted vaginal delivery rate	24	27.9	29	14.2	p < 0.001	11	13.4	83	9.9	NS
Admission to neonatal unit for poor Apgar score at birth	2	5.5	2	1.0	NS	0	0	4	0.5	NS

the control group. The influence of oxytocin on the incidence of fetal distress and operative delivery rates in the two groups was studied.

Results

During the period of study, 2238 cases satisfied the study criteria; in 1993 (89.1%) cases labor was established within the first 24 hours. In 1041 of these cases, the patients were admitted in the first 6 months of our study and formed the control group; 245 (10.9%) were diagnosed as being in spurious labor. A total of 168 (7.5%) were established in labor after 24 hours but within 7 days of the first admission and formed our study group.

The incidence of fetal distress was significantly higher in the spurious labour group (16.7%) than in the normal labor group (3.8%) (Table I). Those with a reactive admission test had a significantly lower incidence of fetal distress when compared with those with an equivocal or ominous admission test in both groups of patients (Table II). However, fetal distress was not uncommon in the spurious labor group even if the test was reactive, which suggests that the test is of little value in this group of patients. In the spurious labor group the positive predictive value of an abnormal admission test was 37.5%; the positive predictive value of a normal admission test was 85.5%. The corresponding figures for the test in the control group were 15.3% and 96.8%.

Obstetric interventions such as cesarean section, operative vaginal delivery, and augmentation of labor were significantly more common in the spurious labor group than in the control group (Table III). The incidence of induction of labor in the spurious labor group was 15.5%. Thus labor was induced or augmented in more than half the cases of spurious labor (51.2%). The general induction rate in our unit during the period of study was 5.8%.

The use of oxytocin may have influenced the incidence of fetal distress and operative deliveries in the spurious labor group and the control group. In the spurious labor group 86 (51.2%) of 168 patients received oxytocin (26 or 15.5% for induction and 60 or 35.7% for augmentation of labor) compared with 205 (19.7%) patients with augmented labor in the control group. Fetal distress occurred in 22 (25.6%) of 86 who received oxytocin in the spurious labor group compared with 18 (8.9%) of 205 patients in the control group ($p < 0.001$) (Table IV). In the oxytocin group cesarean section and instrument-assisted delivery were also significantly more common among patients with spurious labor than among control subjects. Fetal distress was also significantly more common in the spurious labor group than in the control group even if oxytocin was not given (Table IV).

Comment

The endocrine mechanism that initiates labor is related to an intrinsic balance of different hormones from both the maternal and fetal pituitary[5]: steroids, estrogen, progesterone, prostaglandins, and oxytocin. There is no anatomic, physiologic, or pharmacologic evidence of a "pacemaker" in the uterus to coordinate uterine function, but there appears to be an intrinsic pattern of uterine contractile rhythm for each patient.[6]

A proportion of patients may have difficulty in getting established in labor because of a slight imbalance in the endocrine milieu or because of a lack of neuromuscular coordination in the myometrium, resulting in cases of spurious or false labor. The absence of fundal dominance or predominance of the mid zone of the uterus can lead to painful contractions without progression into established labor, and these cases have the clinical appearance of spurious labor. Such problems have been documented by the use of multichannel tocotransducers placed from the fundus downward.[7]

A high incidence of fetal distress (16.7%) and operative interventions (41.0%) indicates that patients with spurious labor constitute a high-risk group. This finding has not been reported previously. In analyzing the results we looked for contributing factors such as induction and augmentation of labor that may be associated with an increased incidence of fetal distress and operative deliveries. Although an induction rate of 15.5% and an augmentation rate of 35.7% are not uncommon in modern obstetric practice,[8,9] in this specific population with spurious labor they seem to contribute significantly to a higher incidence of fetal distress and operative deliveries (Table IV). The incidence of fetal distress was higher in the spurious labor group than in the control group even in the subpopulation who did not have oxytocin, which indicates the high-risk nature of this group. However, there were no differences in the operative delivery rate when oxytocin was not used.

In this study the value of the admission test as an instrument to select cases more at risk was evaluated. The admission test is based on the hypothesis that uterine contractions may serve as a functional stress to the fetus and reveal fetal distress. In a previous study[4] the admission test was found to be useful in the detection of fetal distress on admission and also to be of some predictive value of fetal well-being during the next few hours of labor in cases where delivery occurred within 24 hours of admission. In this study the incidence of fetal distress was significantly higher in patients who had an equivocal or ominous trace than in those who had a reactive admission test in both the spurious labor and the normal labor groups. However, a fairly high proportion of patients with reactive admission tests in the spurious labor group showed evidence of fetal distress (14.4%); the positive predictive value of a normal test was only 85.5% compared with 96.8% in the control group. These results, when properly interpreted,[10] indicate that this test is of limited value for patients with spurious labor.

This preliminary study suggests that patients with spurious labor constitute a special category needing careful monitoring since fetal distress appeared frequently in subsequent labor. Since there seems to be an association between the high operative delivery rate and fetal distress with the use of oxytocin, further studies are needed to evaluate a proper policy of management.

REFERENCES

1. Friedman EA. Dysfunctional labour. In: Cohen RW, Friedman EA, eds. Management of labor. Baltimore: University Park Press, 1983:11-23.
2. Quinn MA, Murphy AJ, Gallaghar J. "Spurious" labour—does it matter? Aust NZ J Obstet Gynaecol 1984;21:167.
3. Beazley JM, Lobb MO. The management of spontaneous labour. In: Beazley JM, Lobb MO, eds. Aspects of care in labour. London: Churchill Livingstone, 1983:27-51.
4. Ingemarsson I, Arulkumaran S, Ingermarsson E, TambyRaja RL, Ratnam SS. Admission test: a screening test for fetal distress in labor. Obstet Gynecol 1986;68:800-6.
5. Fuchs A-R, Fuchs F. Endocrinology of human parturition: a review. Br J Obstet Gynaecol 1984;91:948.
6. Schulman H, Romney SL. Variability of uterine contractions in normal human parturition. Obstet Gynecol 1970;36:215.
7. Reynolds SRM, Hellman LM, Bruns P. Patterns of uterine contractility in women during pregnancy. Obstet Gynecol 1970;36:215.
8. McNaughton MC. Induction of labour: the case for a high rate. In: Beard RW, Paintin DB, eds. Outcome of obstetric intervention in Britain. London: Royal College of Obstetricians and Gynaecologists, 1980:137-49.
9. O'Driscoll K, Folley M, MacDonald D. Active management of labor as an alternative to cesarean section for dystocia. Obstet Gynecol 1984;63:485.
10. Stempel LE. Eenie, meenie, minie, mo . . . what do the data really show? AM J OBSTET GYNECOL 1982;144:745.

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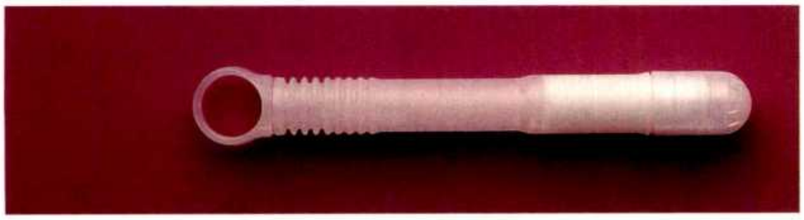

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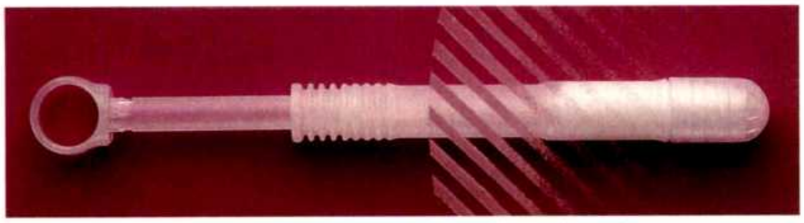

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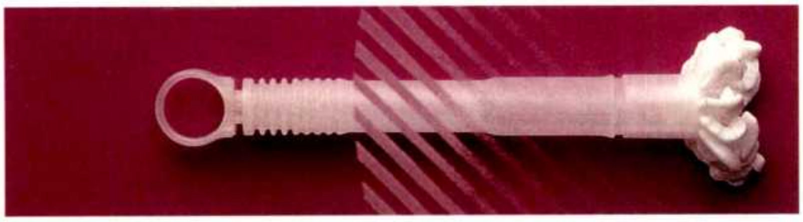
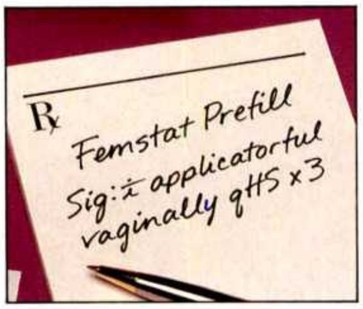


3. Push ring all the way forward to deliver medication. Withdraw applicator and discard.



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The anatomic distribution of cervical adenocarcinoma in situ: Implications for treatment

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Toronto, Ontario, Canada

The anatomic distribution of cervical adenocarcinoma in situ was ascertained in 23 cases of adenocarcinoma in situ, 10 of which also had "early" invasive adenocarcinoma. The adenocarcinoma in situ involved both surface and gland epithelia in all cases, involved a variable number of quadrants, involved glands beneath the transformation zone in about two thirds of cases, was multifocal only occasionally, extended up the endocervical canal for a variable distance (up to 30 mm), and was associated with squamous dysplasia in about half of cases. From this topographic outline of adenocarcinoma in situ, it is recommended that if cervical conization is chosen as conservative therapy for adenocarcinoma in situ the "cone" be cylindrical in shape, to include the transformation zone and deep glands, and extend at least 25 mm up the endocervical canal. (AM J OBSTET GYNECOL 1987;157:21-5.)

Key words: Adenocarcinoma in situ of cervix, cervical conization, adenocarcinoma of cervix

Cervical adenocarcinoma in situ is the putative precursor lesion of invasive adenocarcinoma of the cervix.[1-6] Although adenocarcinoma in situ is uncommon, patients with this disease are frequently young and may present difficult therapeutic choices between hysterectomy and cervical conization only. In our study, the anatomic distribution of cervical adenocarcinoma in situ and glandular dysplasia is assessed. The implications of these findings in the use of conization as treatment of preinvasive glandular neoplasia are discussed.

Material and methods

A review of the pathologic diagnostic index at the Toronto General Hospital from January 1980 to April 1986 yielded 57 cases of adenocarcinoma involving the cervix. Of these cases, 24 were primary endocervical adenocarcinomas in which the following criteria were met: (1) The entire cervix of the hysterectomy specimen or the conization specimen had been submitted for histologic examination and was available for microscopic review; (2) the diagnosis was either adenocarcinoma in situ and/or glandular dysplasia or invasive adenocarcinoma involving less than half of the cervical circumference. Three more cases of adenocarcinoma in situ were identified in the consultation files of the Canadian Reference Centre for Cancer Pathology. These 27 cases formed the basis of this study.

The age, presenting symptoms, and colposcopic findings of patients with adenocarcinoma in situ were recorded. The treatment of patients with adenocarcinoma in situ only was recorded. Follow-up of these patients was concluded in May 1986, yielding a follow-up period of between 2 and 66 months.

The histologic slides from the radial sections of all hysterectomy and conization specimens and any previous cervical biopsies were then reviewed in all 27 cases by a single pathologist (T. J. C.); in cases of adenocarcinoma in situ, the resection margins of any conization specimen were recorded.

The morphologic criteria for adenocarcinoma in situ have been described and defined by other authors.[1-8] In brief, the diagnosis of adenocarcinoma in situ is based on both architectural and cytologic features. In contrast to the normal, regular endocervical epithelium with its basal nuclei, neoplastic endocervical epithelium is multilayered or pseudostratified (Fig. 1). Glands may exhibit tight, compact glandular clusters ("tunnel clusters")[4] and intraglandular epithelial "bridges." The cellular epithelium shows packed nuclei oriented perpendicular to the epithelial basement. Cytologically, a marked increase in the nuclear:cytoplasmic ratio, increased nuclear hyperchromatism, and variable degrees of nuclear pleomorphism and mitotic activity are seen. Cellular boundaries are indistinct (Fig. 2). Lesser degrees of these abnormalities have been labeled columnar cell dysplasia by some authors.[4] For the purposes of this study no grading of columnar cell abnormality was attempted. Instead endocervical epithelium

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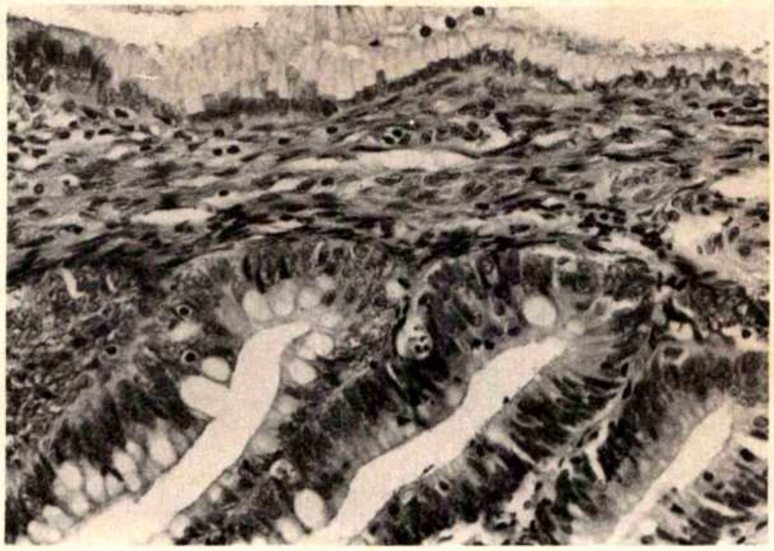

Fig. 1. Normal endocervical gland *(top)*, with its regular, mucinous epithelium and basal nuclei, and adenocarcinoma in situ involving three glandular clefts *(bottom)* with a disorderly, cellular columnar epithelium. (Original magnification ×370.)

Table I. Circumferential extent of adenocarcinoma in situ by quadrant

	Patients with adenocarcinoma in situ only	*Patients with in situ and invasive adenocarcinoma**
One quadrant	2	1
Two quadrants	4	2
Three quadrants	4	3
Four quadrants	3	1

*The extent of quadrant involvement could not be determined in all patients with adenocarcinoma in situ and invasive carcinoma.

exhibiting the above mophologic abnormalities was termed adenocarcinoma in situ.

The following histologic parameters of adenocarcinoma in situ were studied in all cases with or without an associated invasive component with the use of all available cervical material from any case (biopsy, cone, and hysterectomy specimens):

1. The presence or absence of endocervical surface and gland involvement was evaluated.

2. The number of quadrants involved by adenocarcinoma in situ was ascertained.

3. Involvement of endocervical glands beneath the cervical transformation zone was studied. The transformation zone of the cervix is delimited by the functional squamocolumnar junction and the original squamocolumnar junction, as indicated by the "last" remaining endocervical gland beneath the squamous epithelium.[9] Endocervical glands remain beneath the maturing transformation zone and if they were involved by adenocarcinoma in situ the case was labeled as "adenocarcinoma in situ present beneath the transformation zone." Although all cervices obtained at hysterectomy showed the entire transformation zone, some conization specimens did not, and consequently study of these specimens may underestimate the involvement of endocervical glands beneath the transformation zone by adenocarcinoma in situ.

4. The focality was analyzed. If adenocarcinoma in situ was present only in contiguous radial sections of the cervix, even if it was only focal in some slides, the case was labeled "unifocal." On the other hand, if a complete radial cervical section showed no adenocarcinoma in situ and separated two areas of adenocarcinoma in situ, then the case was termed "multifocal." Since a single section through a winding geographic pattern of adenocarcinoma in situ may yield several, apparently separate foci in a single slide, this finding was not accepted as "multifocal" adenocarcinoma in situ.

5. The highest focus of involvement by adenocarcinoma in situ of the endocervical canal was measured (a) in hysterectomy specimens from the maximal convexity of the cervix to the highest point of involvement of the endocervical canal or glands or (b) in cervical conization specimens from the exocervical resection margin to the highest point of involvement in the endocervical canal or glands. All measurements were taken from histologic sections of a paraffin block of 10% formalin-fixed tissue. No cervical specimen in this series was from a gravid uterus.

6. Presence or absence of any associated squamous dysplasia or carcinoma in situ was studied.

7. The type of adenocarcinoma in situ was ascer-

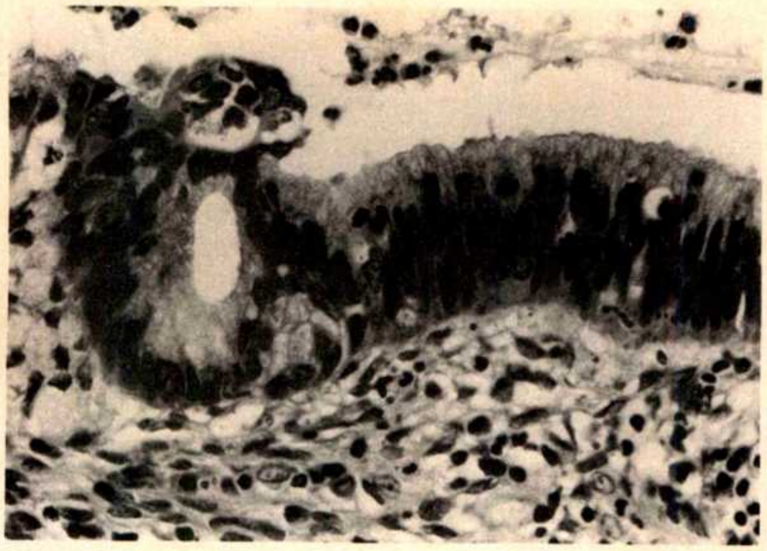

Fig. 2. Adenocarcinoma in situ. Epithelial stratification and "bridging" with nuclear hyperchromatism, pleomorphism, and mitotic activity. (Original magnification ×590.)

tained. Gloor and Ruzicka[7] have defined two types of adenocarcinoma in situ. Type I is described and illustrated in Figs. 1 and 2, and type II is characterized by many of the same architectural and cytologic abnormalities *plus* abundant cytoplasmic mucin.[7]

Results

The 27 cases in this study were divided into three groups. In 13 patients the cervix showed adenocarcinoma in situ only, without any stromal invasion. The patients were aged 26 to 64, with a mean age of 36.4. In 10 patients the cervix showed adenocarcinoma in situ accompanied by invasive adenocarcinoma. These patients were aged 32 to 55, with a mean age of 40.8. Finally, in four patients the cervix showed invasive adenocarcinoma without adenocarcinoma in situ, and these cases were deleted from the study of the topography of adenocarcinoma in situ.

One of 10 patients with adenocarcinoma in situ only and an available history had symptoms (postcoital bleeding) and a second patient had postmenopausal bleeding due to endometrial hyperplasia. The remaining eight patients had no symptoms at all, the majority of them having an abnormal cervicovaginal Papanicolaou smear. In contrast, five of seven patients with adenocarcinoma in situ and invasive adenocarcinoma had postcoital bleeding (the remaining two being asymptomatic). Although colposcopic abnormalities attributable to associated squamous dysplasia were found in the group with adenocarcinoma in situ, no specific colposcopic abnormality relating to adenocarcinoma in situ was detected.

In the 23 patients with adenocarcinoma in situ of the cervix, with or without any associated invasive adenocarcinoma, detailed studies were made. In all 23 patients adenocarcinoma in situ involved both the surface and glandular endocervical epithelium, often with involvement of the deepest glandular clefts. The quadrants involved by adenocarcinoma in situ were listed (see Table I). In 15 of the 23 patients, adenocarcinoma in situ involved glands beneath the cervical transformation zone. In seven patients, adenocarcinoma in situ was not identified beneath the cervical transformation zone. In one case, no transformation zone was present in the conization specimen.

In 15 of the 23 patients, the adenocarcinoma in situ was unifocal. In three patients, involvement was multifocal. In five patients, the focality of adenocarcinoma in situ could not be determined since the sequence of the radial sections of the cervix could not be established.

The highest focus of cervical involvement by adenocarcinoma in situ was measured in 19 cases, and the results are presented in Table II. In four cases, this parameter could not be determined because adenocarcinoma in situ was present in a biopsy specimen only

Table II. Highest focus of cervical involvement by adenocarcinoma in situ*

Distance (mm)	*No. of patients*
0-4.9	2
5-9.9	6
10-14.9	6
15-19.9	1
20-24.9	3
25-29.9	1

*Measured from cervical portio vaginalis on hysterectomy specimens or exocervical margin of cervical cone.

(one patient) or associated invasive adenocarcinoma that had spread upward at the upper endocervical margin of adenocarcinoma in situ obliterated the native mucosa (three patients).

Eleven of the 23 patients with adenocarcinoma in situ had squamous dysplasia or carcinoma in situ in addition to the glandular lesion. Although foci of type II adenocarcinoma in situ were occasionally seen, in none of the 23 cases was this type of adenocarcinoma in situ the predominant one.

All 10 patients with adenocarcinoma in situ and invasive adenocarcinoma were treated with radical hysterectomy and pelvic lymph node dissection. The 13 patients with adenocarcinoma in situ and no invasive adenocarcinoma had a variety of treatments.

Seven patients were treated by hysterectomy after a cervical conization (six patients) or by cervical biopsy (one patient); the five patients with follow-up have had no further problems with respect to cervical disease. Follow-up ranges from 2 to 66 months, with a mean of 18 months. The six hysterectomy specimens from patients who previously underwent conization did not show any residual adenocarcinoma in situ. Four of these conization specimens had shown no involvement of the cone resection margins, while one conization specimen showed adenocarcinoma in situ focally involving the exocervical resection margin. In the remaining patient the cone resection margins could not be determined.

In one patient, adenocarcinoma in situ was discovered incidentally at a hysterectomy for uterine bleeding due to endometrial hyperplasia; she also remains well. In two patients adenocarcinoma in situ was diagnosed on a cervical conization specimen, but no further follow-up was available. Three patients were treated by cervical conization only. All remain clinically well at 5, 21, and 25 months of follow-up; colposcopic and cytologic evaluations remain negative. In two of these three patients, adenocarcinoma in situ was not found on the cone resection margins, although one patient had a positive exocervical cone margin.

Comment

The mean age of our patients with adenocarcinoma in situ only was 36.5, which is comparable to the range of 33 to 42 previously reported.[3-7, 10] The mean age of our patients with adenocarcinoma in situ and "early" invasive adenocarcinoma was about 4 years older. Bousfield et al.[11] noted that the mean age of patients with "microinvasive" adenocarcinoma was 5 years older than the mean age of patients with adenocarcinoma in situ only.

Most patients with adenocarcinoma in situ only are entirely asymptomatic, as were nine of 10 in our series.[1-3, 5, 6, 10, 12, 13] Nevertheless, some patients with adenocarcinoma in situ have presented with symptoms, such as vaginal discharge, postcoital bleeding, postmenopausal bleeding, and intermenstrual bleeding.[3-6, 13, 14] Some of the reported symptoms may have been due to other genital tract lesions; one of our patients had associated postmenopausal bleeding secondary to endometrial hyperplasia. Nevertheless, the possibility remains that adenocarcinoma in situ may produce symptoms; one of our patients had postcoital bleeding.[14]

The anatomic distribution of adenocarcinoma in situ has not yet been well defined. Our study revealed that in all cases of adenocarcinoma in situ, with or without invasive adenocarcinoma, the neoplastic process involved both the surface and the glandular endocervical epithelium. This finding corroborates previous studies showing this pattern.[1, 4-7, 13] The finding of adenocarcinoma in situ involving glands without surface involvement has been reported rarely.[3] Sparing of the deepest glands of the endocervix by adenocarcinoma in situ was not noted in our series, although others have noted this tendency.[3, 4, 6, 7]

The number of quadrants involved by adenocarcinoma in situ varied from one to four without any predilection for limited or extensive involvement (Table I). The accumulated evidence to date about the circumferential extent of adenocarcinoma in situ has been conflicting, some authors indicating adenocarcinoma in situ to be focal or localized[1, 3, 5] and others reporting adenocarcinoma in situ to be usually extensive.[2, 7] Ostor et al.[6] measured the width of involvement on a "spread-out" conization specimen and found it to vary from 0.5 to 25 mm, with a mean of 12 mm. In our series no correlation exists between the presence of invasive adenocarcinoma and the extent of adenocarcinoma in situ. Specifically, even small areas of adenocarcinoma in situ were associated with invasive adenocarcinoma.

In about two thirds of our patients, adenocarcinoma in situ involved glands beneath the transformation zone. Rarely has mention of adenocarcinoma in situ beneath squamous metaplasia been made previously.[5]

Assessment of multicentricity of adenocarcinoma in situ has not usually been made in past reports.[2, 4, 5, 12-14] Definite multifocality in adenocarcinoma in situ would seem to occur in only a minority of cases.[1, 3, 6] According to our strict definition of multifocality, only three of the 23 patients had multifocal adenocarcinoma in situ. Similarly, Ostor et al.[6] found three of 20 cases to be "multicentric." Although one report found 10 of 14 cases of adenocarcinoma in situ to be "plurifocal" the criteria for this designation were not outlined.[7]

The highest focus of involvement of the endocervical mucosa by adenocarcinoma in situ varied considerably, although in 18 of 19 cases it was less than 25 mm from the portio vaginalis. Another study has found a mean distance of 7 mm, varying from 0.5 to 30 mm.[6]

Adenocarcinoma in situ has previously been found to be associated in at least one half of cases with squamous dysplasia or carcinoma in situ.[3-7, 10, 13] In our series, 11 of 23 patients had an associated squamous abnormality.

Although Gloor and Ruzicka[7] have identified a type II adenocarcinoma in situ of the cervix, in our experience and that of others, this type of adenocarcinoma in situ coexists with the usual type I adenocarcinoma in situ.[6]

Hysterectomy has been advocated as the most appropriate treatment for adenocarcinoma in situ since this procedure removes the entire endocervical mucosa.[5] Nevertheless, several authors[2, 6, 7, 14] have either proposed or used conization as acceptable conservative therapy for cervical adenocarcinoma in situ. From our topographic study we can suggest an optimal cervical conization method if one seeks to excise adenocarcinoma in situ for diagnosis and treatment. The cone should be cylindrical, encompassing all of the transformation zone, if possible, and the depths of the endocervical glands (5 mm from the canal); it should extend parallel to the endocervical canal for at least 25 mm before a 90-degree turn toward the endocervical canal. This yields a flat-based cone. We believe this method should encompass the vast majority of cases of adenocarcinoma in situ (about 95%), including the occasional multifocal cases and has a minimal likelihood of yielding a positive exocervical or endocervical margin. If adenocarcinoma in situ did not involve the margins of the cervical conization specimen, we did not find adenocarcinoma in situ in a subsequent hysterectomy specimen; this result agrees with the findings of others.[6]

We thank the Canadian Reference Centre for Cancer Pathology and Drs. I. Dardick and W. T. E. McCaughey for their encouragement and support and Ms. C. White for her technical assistance.

REFERENCES

1. Friedell GH, McKay DG. Adenocarcinoma in situ of the endocervix. Cancer 1953;6:887.
2. Laughlan SC, Penner DW. Simultaneous adenocarcinoma in situ and epidermoid carcinoma in situ. Cancer 1967;20:2250.
3. Qizilbash AH. In-situ and microinvasive adenocarcinoma of the uterine cervix. Am J Clin Pathol 1975;64:115.
4. Barter RA, Waters ED. Cyto- and histo-morphology of cervical adenocarcinoma in situ. Pathology 1970;2:33.
5. Christopherson WM, Nealon N, Gray LA. Noninvasive precursors of adenocarcinoma and mixed adenosquamous carcinoma of the cervix uteri. Cancer 1979;44:975.
6. Ostor AG, Pagano R, Davoren RAM, Fortune DW, Chanen W, Rome R. Adenocarcinoma in situ of the cervix. Int J Gynecol Pathol 1984;3:179.
7. Gloor E, Ruzicka J. Morphology of adenocarcinoma in situ of the uterine cervix. Cancer 1982;49:294.
8. Boon ME, Baak JPA, Hurver PJH, Overdiep SH, Verdonk GW. Adenocarcinoma in situ of the cervix. Cancer 1981;48:768.
9. Burghardt E, Ostor AG. Site and origin of squamous cervical cancer: a histomorphologic study. Obstet Gynecol 1983;62:117.
10. Kluzak T, Kraus F. Adenocarcinoma in situ of the uterine cervix (AIS): immunohistochemical staining for carcinoembryonic antigen (CEA) and human papillomavirus (HPV) [Abstract]. Lab Invest 1986;54:32A.
11. Bousfield L, Pacey F, Young Q, Krumins I, Osborn R. Expanded cytologic criteria for the diagnosis of adenocarcinoma in situ of the cervix and related lesions. Acta Cytol 1980;24:283.
12. Yamasaki M, Ueda G, Sato Y, Kobayashi Y, Kurachi K. Simultaneous squamous cell carcinoma in situ and early invasive adenocarcinoma of uterine cervix. Acta Obstet Gynaecol Jpn 1975;22:6.
13. Weisbrot IM, Strabinsky C, Davis AM. Adenocarcinoma in situ of the uterine cervix. Cancer 1972;29:1179.
14. Abell MR, Gosling JRG. Gland cell carcinoma (adenocarcinoma) of the uterine cervix. AM J OBSTET GYNECOL 1972;83:729.

Histologic composition of endometrial carcinomas analyzed for steroid receptor content

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Durham, North Carolina

One hundred endometrial carcinoma specimens were analyzed for estrogen receptor content and histologic composition. Benign tissue elements were identified in the majority of tissue sections. Histologic control of endometrial carcinoma specimens submitted for steroid receptor analysis is critical, because biochemical assays of whole tissue homogenates cannot determine receptor localization among tissue components. (AM J OBSTET GYNECOL 1987;157:26-7.)

Key words: Endometrial carcinoma, histologic composition, estrogen receptor content

Several investigators have evaluated clinical and therapeutic significance of estrogen receptor and progesterone receptor content in endometrial carcinomas. We previously reported that tissues from endometrial carcinomas submitted for biochemical estrogen receptor and progesterone receptor analyses require strict histologic control to ensure that assays of binding in the whole-tissue homogenate are representative of the malignancy.[1] The purpose of this study was to evaluate histologic composition of endometrial carcinoma specimens to determine the relative contribution of malignant and nonmalignant tissues to whole-tissue homogenates assayed for steroid receptor content.

Material and methods

One hundred tissue specimens from endometrial carcinomas accessioned between 1980 and 1983 had sufficient tissue for biochemical analysis of steroid receptors and histologic analysis of frozen section from the tissue specimen submitted for steroid receptor analysis. Tissue was obtained by biopsy or from hysterectomy. Specimens were quick frozen after being washed in estrogen receptor assay buffer (0.005 mol/L HEPES, 0.01 mol/L Tris, 0.0015 mol/L EDTA, 0.01 mol/L thioglycerol, and 0.02% sodium azide at pH of 7.4) and maintained at −70° C until steroid receptor analyses were performed. Specimens were then stored in liquid nitrogen or at −70° C (Revco freezer) before histologic analysis.

For each specimen, biochemical estrogen receptor analysis was performed by methods previously described,[2] including multiconcentration titration analysis (dextran–coated charcoal technique) and sucrose density gradient analysis of estrogen binding. For the purpose of this study, saturable binding calculated to have a dissociation constant $<10^{-10}$ by dextran–coated charcoal analysis or binding in the 4S through 8S region by sucrose density gradient analysis was considered the estrogen receptor content of the tissue homogenate.

Histologic analysis was performed on thin (4 μm) sections made with the cryostat from tissue submitted for steroid receptor analysis and stained with hematoxylin-eosin stain. Two observers independently quantitated the visual estimate of tissue composition for each specimen, evaluating proportions of each of the following histologic components: (1) malignant epithelium, (2) benign epithelium, (3) stroma, and (4) myometrium. A consensus tissue composition and histologic grade were generated for each specimen. Histologic differentiation was classified into three grades: grade 1 lesions were composed of well-differentiated glandular elements, grade 2 lesions were intermediate in differentiation, and grade 3 lesions were composed predominantly of sheets of malignant cells with rare gland formation.[2]

Results

Thirty-seven specimens had grade 1, 37 had grade 2, and 26 had grade 3 lesions. Estrogen receptor content of tissue homogenate was associated with histologic grade. Median estrogen receptor content of differentiated (grades 1 and 2) lesions was 180 fmol/mg

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protein (median fiftieth percentile 140 to 220 fmol/mg protein) versus 90 fmol/mg protein (median fiftieth percentile 0 to 130 fmol/mg protein) for undifferentiated (grade 3) lesions (Mann-Whitney U test, $z = 4.32$; $p < 0.001$).

Myometrial and stromal elements were identified in the majority of tissue specimens whereas a few had significant benign epithelial components (Fig. 1). Malignant epithelial elements accounted for <50% of sections in 20% of specimens whereas only 5% of specimens were composed entirely of malignant epithelial elements (Fig. 1). The distribution of malignant epithelial elements did not differ significantly by histologic grade, accounting for a median 60% of specimen for grades 1 and 2 lesions and 55% of specimen for grade 3 lesions.

Comment

Histologic control of endometrial carcinoma specimens submitted for biochemical assays of steroid receptor content is critical because biochemical assays are performed on tissue homogenates and do not yield information concerning histologic findings of tissue analyzed for steroid receptor. Conventional histologic analysis can be used to exclude specimens containing a paucity of carcinoma[1]; however, at best this can take the form of arbitrarily assigning an acceptable threshold for carcinoma content (e.g., 20%) and excluding specimens with malignant epithelial component less than this threshold. Furthermore, knowing the histologic composition of endometrial carcinoma specimens does not allow assessment of the relative contributions made by individual histologic components toward the total steroid receptor content as measured by biochemical assays of tissue homogenate.

In this study histologic components other than malignant epithelium accounted for the majority of tissue analyzed for receptor content in 20% of specimens. Only a few specimens were composed entirely of malignant epithelial elements (Fig. 1). Cumulative distribution functions of histologic composition were similar among all histologic grades. Therefore, even in poorly differentiated (grade 3) lesions the potential exists for significant contribution by nonmalignant tissues to total receptor content as measured by standard biochemical assays.

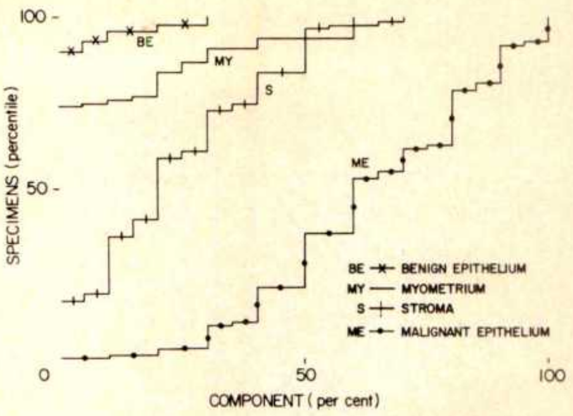

Fig. 1. Cumulative distribution function of histologic components identified in endometrial carcinoma specimens analyzed for steroid receptor content. This plot allows estimation of the distribution of each histologic component, as well as a visual comparison of the distribution of all components simultaneously (e.g., 20% of specimens composed of <50% malignant epithelium and 20% of specimens composed of >50% stroma).

In breast carcinomas this issue is not important because adjacent normal breast tissues contain low levels of receptor. However, normal endometrial and uterine tissues contain significant steroid receptor. Receptor content of normal endometrium may vary from different regions within the uterus and under varying hormonal environments. The definitive method for histologic control and localization of steroid receptor content in endometrial carcinomas awaits the development of sensitive and specific immunohistologic techniques. Immunohistologic techniques for steroid receptor analysis must be validated by comparison with both standard biochemical steroid receptor assays and the biologic behavior of endometrial carcinomas, such as prognosis after primary therapy and response to hormonal therapy.

REFERENCES

1. Soper JT, McCarty KS Jr, Creasman WT, McCarty KS Sr. Histologic control of biochemical steroid receptor analysis in endometrial carcinomas. AM J OBSTET GYNECOL 1985; 153:520-3.
2. McCarty KS Jr, Barton TK, Fetter BF, et al. Correlation of estrogen and progesterone receptors with histologic differentiation in endometrial adenocarcinoma. Am J Pathol 1979;97:171-84.

Diagnostic imaging of gynecologic tumors with the monoclonal antibody 791T/36

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One hundred twenty-three patients with suspected primary or recurrent gynecologic cancer were imaged after infusion with radiolabeled antitumor monoclonal antibody (791T/36). Overall sensitivity of tumor detection was 87% and specificity was 75%. Indium 111– and iodine 131–labeled antibody were compared, the former yielding a higher specificity for tumor detection. This antibody shows potential for routine tumor detection, particularly in cases of suspected recurrent cancer, but the development of antimouse antibodies is a drawback to this technique still to be overcome. The specific targeting of cytotoxic agents with this antibody is in progress. (Am J Obstet Gynecol 1987;157:28-34.)

Key words: Diagnostic imaging, gynecologic tumors, monoclonal antibody 791T/36

Monoclonal antibodies were first produced in 1975 by the cell fusion technique of Kohler and Milstein.[1] The purity and specificity that can be obtained with monoclonal antibodies have suggested that they may play an important role in tumor detection and potentially in the ultimate goal of targeting antitumor agents. Tumor localization by a radiolabeled monoclonal antibody was first described in the United Kingdom by Farrands et al.[2] in patients with colorectal cancer. The murine monoclonal antibody employed in this study was raised against a tumor-associated antigen with an in vitro cell line of osteogenic sarcoma as the immunogen by the Cancer Research Campaign Laboratories at Nottingham University, and it has been designated 791T/36. Subsequent studies have shown this antibody to localize primary osteogenic sarcoma,[3] and colonic[4] and ovarian[5] cancer. The imaging studies involving gynecologic cancers have now been expanded.

Material and method

The monoclonal antibody 791T/36, an IgG2b mouse immunoglobulin, was isolated from hybridoma ascitic fluid by protein A affinity chromatography. Purified antibody was then radiolabeled with either iodine 131 via an iodogen method or indium 111 after the label was conjugated to diethylenetriaminepentaacetic acid dianhydride. Labeled preparations were then diluted in 10 ml of saline for injection and sterilized by terminal filtration. Either ^{131}I or ^{111}In, 40 to 70 mEq, was conjugated with 1 to 2 mg of antibody. A subcutaneous test dose of one tenth of the imaging dose was given before infusion to test for anaphylaxis. Patients receiving ^{131}I-labeled antibody also required oral potassium iodine throughout the study to block thyroid uptake of free iodine. In addition, blood pool subtraction techniques were required for ^{131}I studies[6] to correct for the nontumor distribution of labeled antibody. This was not necessary for indium-labeled antibody or for indium-labeled preparations.

Imaging was performed between 48 and 72 hours after the antibody was administered. Images were acquired with an IGE 400T large-field-of-view gamma camera and stored by computer in a 64 × 64 or 128 × 128 element matrix. Anterior and posterior views of

Table I. Tumor distribution

Tumor site	*Antibody indium 111*	*Radiolabel iodine 131*	*Total*
Ovary			
Primary	7	7	14
Recurrent	12	36	48
Benign	12	4	16
Cervix			
Primary	1	8	9
Recurrent	4	9	13
Corpus			
Primary	2	4	6
Recurrent	0	4	4
Benign	3	5	8
Nongynecologic			
Primary	2	3	5
Recurrent	0	3	3
Total	43	83	126

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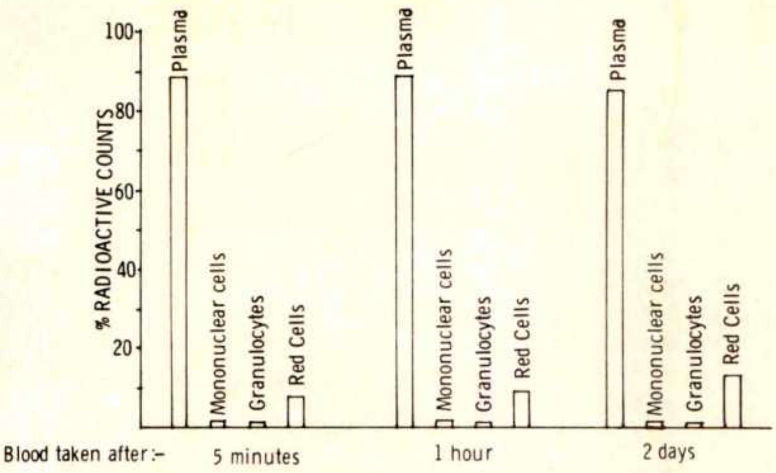

Fig. 1. Blood distribution of radioactivity after injection with iodine-labeled 791T/36. Radioactivity in each of the fractions is expressed as a proportion of the recovered counts.

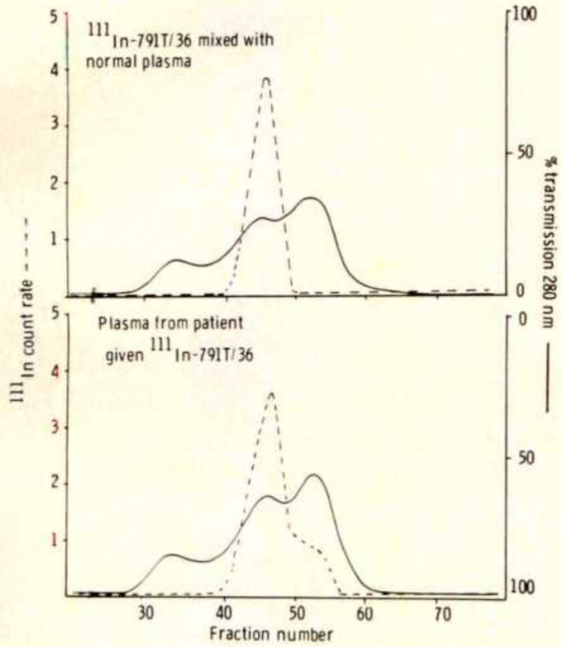

Fig. 2. Gel filtration chromatography of ^{111}In-labeled 791T/36 preparation before injection and in patient's blood 72 hours after injection.

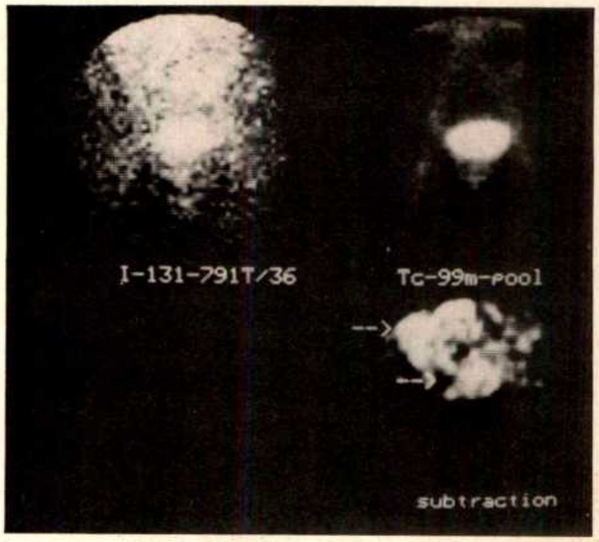

Fig. 3. Gamma camera images of the anterior pelvis 48 hours after injection of ^{131}I-labeled 791T/36. An area of increased uptake on the right side of the pelvis seen on the subtracted image corresponds to the tumor site.

the pelvis, abdomen, and thorax were recorded. ^{111}In-labeled antibody was more suitable for emission tomography than ^{131}I, and benefits of the additional information with regard to tumor location were assessed. Tomography was performed by acquiring data throughout a 360-degree rotation of the gamma camera by use of both of the principal photopeaks of ^{111}In as described previously.[7] In addition, the specimens from patients undergoing tumor resection were imaged.

Tissue samples were taken from the tumor and nontumor areas. After the samples were weighed the radioactivity was measured in a well scintillation counter. The count rates were then expressed as a percentage of the administered dose.

One hundred twenty-three patients were included in the present study. In all cases the clinician had a strong index of suspicion of primary or recurrent gynecologic malignancy (Table I). In those patients with clinically suspected recurrent tumor, a second-look laparoscopy was carried out to confirm or negate the immunoscintigraphy findings. Where laparoscopic findings were inconclusive magnetic resonance scans were carried out. Although recently introduced, this latter technique has shown potential in imaging suspected recurrent ovarian cancer.

Table II. Tumor detection

		Indium 111				*Iodine 131*			
		True		*False*		*True*		*False*	
Tumor	*No. of studies*	+	–	+	–	+	–	+	–
Ovary									
Primary	14	7	—	—	0	7	—	—	0
Recurrent	48	5	5	1	1	20	14	2	0
Benign	16	—	10	2	—	—	1	3	—
Cervix									
Primary	8	1	—	—	—	4	—	—	3
Recurrent	12	3	1	0	0	4	2	0	2
Corpus									
Primary	5	1	—	—	1	3	—	—	0
Recurrent	4	—	—	—	—	2	2	0	0
Benign	8	—	1	2	—	—	3	2	—
Nongynecologic									
Primary	5	1	—	—	1	2	—	1	—
Recurrent	3	—	—	—	—	3	0	0	0

Total number of patients = 123: indium N = 43 and iodine N = 80 (three patient studies were not completed because of technical difficulties).

Results

The procedure was well tolerated in all patients receiving the infusion for the first time. The antibody survived in circulation, predominantly (90%) in plasma, with little binding to mononuclear cells or granulocytes (Fig. 1). Gel filtration chromatography showed plasma-borne radiolabel predominantly as monomonic immunoglobulin G, although with ^{111}In as the radiolabel some labeled components of lower molecular weight, probably transferrin, were seen (Fig. 2).

The results of the imaging studies involving 123 patients are given in Table II. Three patients were excluded owing to technical difficulties. It is evident that 791T/36 with either ^{131}I or ^{111}In radiolabel has both a high sensitivity and specificity (87% and 75%, respectively) for the external detection of gynecologic cancer.

Fig. 3 is an example of a ^{131}I-labeled antibody study of a patient with a clinically palpable mass in the right iliac fossa suspected to be a recurrence of a previously resected ovarian tumor. Images before and after subtraction of the technetium 99m–labeled blood pool image are shown. On the right side of the pelvis there is an increased area of antibody uptake, corresponding to the site of the suspected recurrence. This was subsequently confirmed by laparoscopy as a large recurrent ovarian adenocarcinoma. Normal uptake of radioiodine was seen in the thyroid, gastrointestinal mucosa, kidneys, and bladder.

In contrast ^{111}In-labeled antibody had different in vivo biodistribution, with uptake of radiolabel into the liver, spleen, bone marrow, and occasionally the adrenal glands. Excretion of ^{111}In into the bladder was not a problem. Accumulation of the ^{111}In-labeled antibody was seen in bowel in a small number of patients, although tumor uptake could generally be seen above the background activity in normal bowel.

Fig. 4, *left*, is an example of a ^{111}In study. This shows images of a 74-year-old woman with malignant granulosa cell tumor. There is an increased area of uptake centrally in the pelvis corresponding to the tumor site. Emission tomographic studies aided the definition of tumor site (Fig. 4, *right*).

The sensitivity and specificity results for ovarian, cervical, and corpus tumors are in Table III.

There were sufficient numbers of patients with ovarian tumors to split into "iodine" and "indium" groups. For ovarian tumors ^{111}In had a sensitivity of 92% and specificity of 82%. In contrast, ^{131}I appeared to be more sensitive (100%) for the detection of ovarian malignant tissues but was less specific (75%). There were no false negative test results with ^{131}I studies but five false positive results. These were probably due to artifacts resulting from the subtraction technique.[9] At histopathologic examination two of the benign ovarian cysts were found to be ovarian teratomas, both of which contained thyroid tissue, which might account for the concentration of ^{131}I that gave false positive results. The ^{111}In-labeled antibody appeared to have greater specificity in the discrimination between malignant and benign ovarian lesions.

In the group of 48 patients who were investigated for the presence of recurrent ovarian cancer, 25 had the disease. Thirteen of these had a clinically palpable mass, nine had recurrent disease confirmed by a second-look laparotomy or laparoscopy, and in three patients a pelvic magnetic resonance scan was performed. Nineteen of the 48 patients had negative results of examination; 10 of these underwent a second-

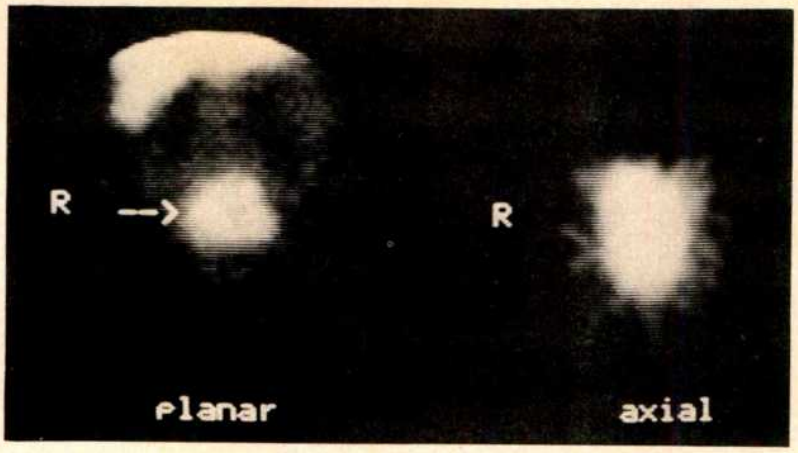

Fig. 4. Gamma camera planar *(left)* and tomographic images of a patient with a primary malignant granulosa cell tumor of the ovary 72 hours after administration of ^{111}In-labeled 791T/36.

look laparoscopy, and four had magnetic resonance imaging of the pelvis. Five patients were thought to be clinically free of the disease at the time of immunoscintigraphy. There were three false positive results in this group; one patient had laparotomy, and the tissue removed contained mainly fibrous elements with no active tumor. In two cases where ^{131}I imaging gave false positive results, the laparoscopies were technically difficult owing to the presence of adhesions obscuring the view of the pelvis. The subsequent well-being of these patients over 3 years indicated they were in fact free of tumor at the time of the examination. These latter two examinations were performed before magnetic resonance imaging was available to verify the results.

Fig. 5 is an example of ^{111}In study of a patient with a Stage IV cervical carcinoma. Two extensive areas of increased uptake corresponding to the large tumor masses are demonstrated in the center of the pelvis. For cervical cancer there was a lower sensitivity when either an ^{111}In- or ^{131}I-labeled monoclonal antibody was employed. There were five false negative results; three of these patients had a large, clinically palpable mass confirmed as malignant cervical carcinoma by biopsy.

Fig. 6 represents a ^{131}I-labeled antibody study of a patient with a recurrent endometrial adenocarcinoma, demonstrating a focal area of increased uptake in the pelvis corresponding to tumor in the uterus. In this group of patients, five who were clinically suspected of having a pelvic malignancy had, at operation, confirmed leiomyomas. Four of these had false positive results, two with ^{131}I- and two with ^{111}In-radiolabeled antibody.

Resected specimens showed increased activity in the tumor, both when on imaging and when the tissue radioactivity was assayed. Fig. 7 shows a ^{111}In study of 26-year-old woman with a malignant cystadenocarcinoma of the ovary. In Fig. 7, *A*, an area of increased uptake can be seen on the left side, in what appears to be a large cystic lesion. This is better demonstrated on the scan of the resected specimen (Fig. 7, *B*), where there is marked uptake within a malignant solid area at the periphery of the lesion. The resected operative specimen is shown in Fig. 7, *C*. Fig. 7, *D*, is an example of the tissue radioactivity found in the normal and malignant tissues of this patient. The tumor-to-normal ovary (taken from the contralateral side) uptake ratio of the resected specimens of gynecologic cancer studied was 4:1.

Table III. Sensitivity and specificity

Tumor	*N*	*Sensitivity (Se)*	*Specificity (Sp)*
Ovary	78	97.5	78.9
Cervix	20	65	100
Corpus	17	85	60

Se = T + ve
T + ve F − ve

Sp = T − ve
T − ve F + ve

Comment

A number of studies have now been reported of imaging of patients with ovarian cancer by means of a radiolabeled monoclonal antibody.[9, 10] A pilot study of 12 women with ovarian tumors was previously reported in which murine monoclonal antibody 791T/36 was used.[6] The present article documents our experience with 791T/36 in 123 patients, which includes 40 women with cervical and uterine tumors. No previous study involving radiolabeled monoclonal antibody imaging of this group of tumors has so far been reported.

In ovarian, cervical, and uterine cancer the murine monoclonal antibody 791T/36 has been demonstrated to have a marked affinity for malignant tissue, with a high sensitivity and specificity. The choice of radionuclide for radiolabeling a monoclonal antibody for

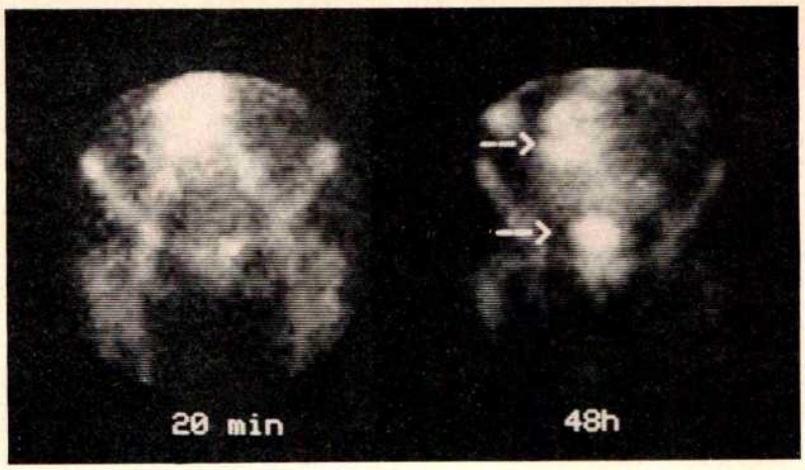

Fig. 5. Gamma camera images of the pelvis at 20 minutes *(left)* and 48 hours after injection of ^{111}In-labeled 791T/36. Two main areas of uptake within the cerivcal carcinoma are demonstrated.

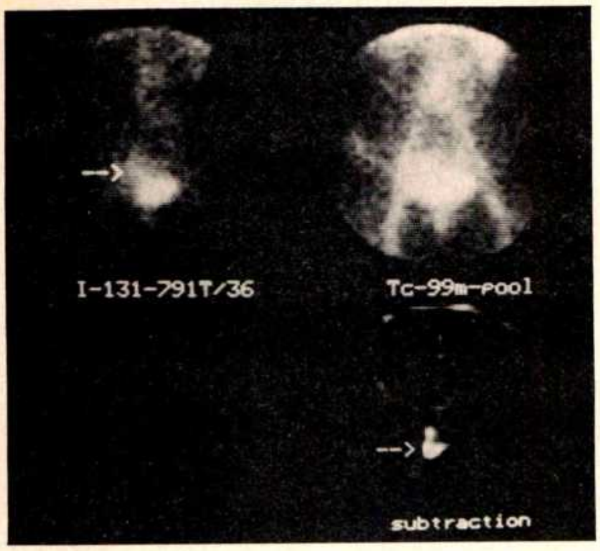

Fig. 6. Gamma camera images of the pelvis 48 hours after injection of ^{131}I-791T/36. A local area of increased uptake can be seen in the center of the pelvis, corresponding to the site of recurrence of an endometrial adenocarcinoma.

imaging purposes is important. ^{131}I has a greater sensitivity in the detection of gynecologic malignancy but is less specific than ^{111}In. There were a number of false positive ^{131}I imaging results that likely resulted from incomplete subtraction of radioiodide in the urinary bladder. ^{111}In, with its superior physical characteristics, appeared to be a more suitable radiolabel for gamma camera imaging. Although it is cleared at the same rate from the bloodstream, the tissue retention of ^{111}In results in a higher tumor-to-normal tissue image contrast.[7] In addition, ^{111}In is more suitable for emission tomography, which leads to a more accurate localization of the antibody uptake site, and this may lead to a reduction in the number of false positive results. Although excretion into the urinary bladder is not a problem with ^{111}In-labeled monoclonal antibody, radioactivity is actively taken into the liver and spleen. This therefore prevents the use of ^{111}In-labeled 791T/36 in the detection of liver metastases. The nonspecific bowel uptake sometimes seen with ^{111}In studies may account for some of the false positive results that occurred with this radiolabel. In the 20 patients with cervical cancer who were imaged, there were five false negative results within the ^{131}I group. In three cases a clinically palpable recurrent tumor was present. The exact reason for this is uncertain. No other false negative test results were observed with ovarian or corpus tumors. This may relate to the squamous cell nature of these tumors, although a previous imaging study in patients with lung tumors has suggested that the tumor detection rate was more related to the size and site of the tumor than to histologic type.[11] The single false negative result within the corpus group was related to the tumor being less than 5 mm in diameter, which is probably beyond the resolution of this technique at the present time.

The diagnostic capability of radiolabeled monoclonal antibodies in gynecologic cancer is evident, but its clinical value in primary disease has to be demonstrated. Information regarding involvement of surrounding pelvic organs is limited. Of more value will be their use in assessment of patients with recurrent tumor. Laparoscopic assessment may be particularly inaccurate in relation to recurrent ovarian cancer.[12] Second-look laparotomies provide more accurate information as to disease extent but are associated with increased morbidity and mortality, and they still must be shown to benefit

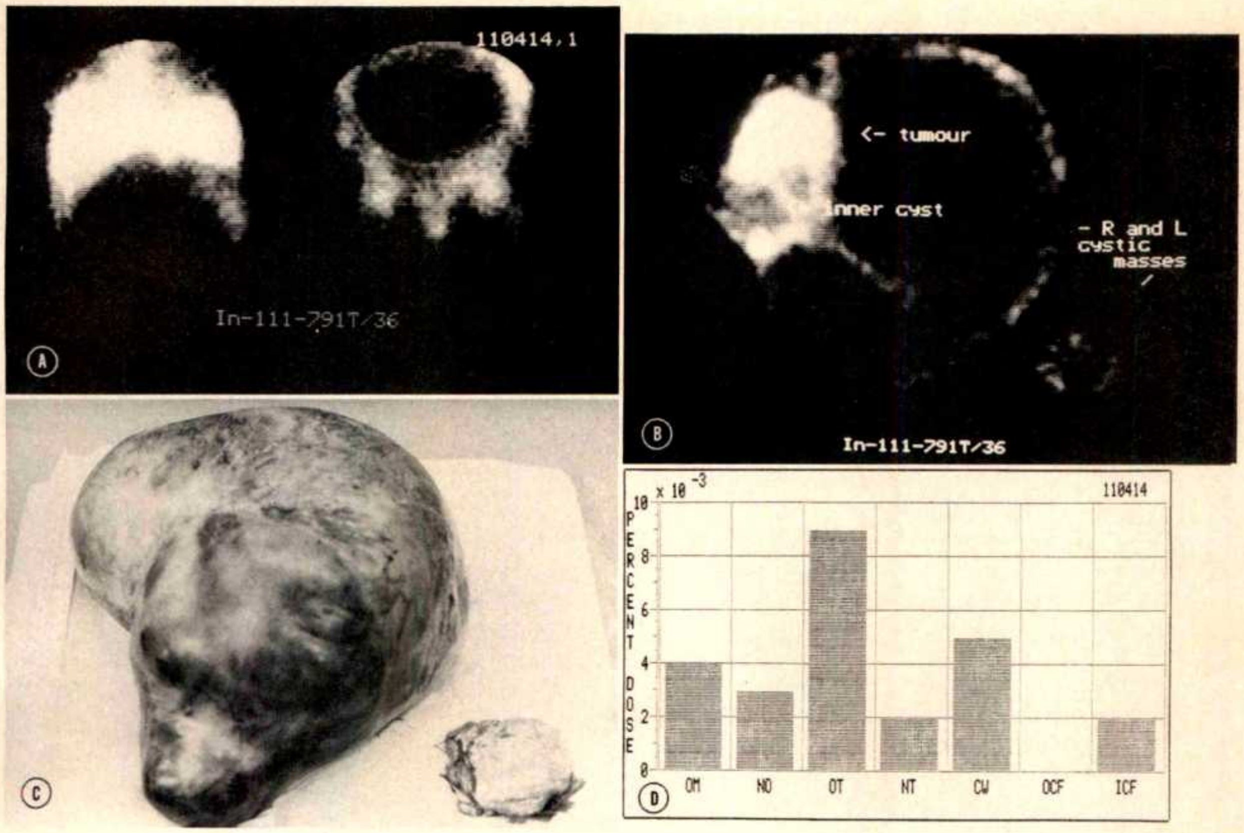

Fig. 7. A, Gamma camera images of the abdomen *(left)* and pelvis *(right)* at 72 hours. Intense uptake is seen in the liver and spleen. There is a central layered cystic area on the pelvic image, with increased uptake at its upper margin. **B,** Gamma camera images of the resected specimen confirm uptake of antibody in an area of solid tumor within the cyst. **C,** Resected specimen including the other smaller cystic ovary. **D,** Plot of radioactivity per gram of resected tissue after dissection of the specimen. *OM,* Omentum; *NO,* normal ovary; *OT,* ovarian tumor; *NT,* normal tube; *CW,* cyst wall; *OCF,* outer cyst fluid; *ICF,* inner cyst fluid.

the patient.[13] Magnetic resonance imaging is a new radiologic technique that, owing to its unique tissue differentiation qualities, may have an important role in evaluating patients with recurrent pelvic tumors, but again this must be determined.

Sixty-five patients in our study group were imaged in order to ascertain whether they had recurrent gynecologic cancer. In this group there was an overall label sensitivity of 92% and a specificity of 88%. Therefore this monitoring technique would appear to be useful in conjunction with other techniques that provide more anatomic demonstrations of the tissues. As an alternative to laparoscopy it would be necessary to administer an imaging dose of antibody on a regular basis, raising the question of the development by the patient of antimouse antibodies. In our group of 65, nine patients received two infusions, three received three, and two patients four infusions. Patients receiving up to three ^{111}In-labeled antibody infusions showed increased uptake of radiolabel into the spleen, which was thought to represent splenic clearance of patient anti-antibody complex.[11] This may affect the result of the scan, although in several of the cases other evidence of tumor absence or presence confirmed the result of the investigation. In one patient who received a fourth infusion, a systemic reaction occurred with a grand mal epileptic seizure, despite a negative skin test. A blood sample taken at this time demonstrated the formation of anti-antibody immune complexes.

Anti-antibody production has been shown to continue for up to 10 months after a single injection of radiolabeled 791T/36 antibody.[14] The formation of these antimouse antibody responses may severely limit the use of immunoscintigraphy for repeat diagnostic imaging.

Monoclonal antibodies have also been proposed for use in the targeting of cytotoxic agents. This would increase the drug concentration at the tumor site and reduce normal tissue toxicity. The use of radioisotope-labeled monoclonal antibodies for therapy has been reported with intraperitoneal injections of ^{131}I-labeled monoclonal antibodies.[15] The 791T/36 antibody conjugates have been constructed with conventional cytotoxic agents and with ricin A chain immunotoxin,[16] and they have been shown to be effective against human tumor xenografts in immunodeprived mice. In gynecologic cancer the antibody 791T/36 has a sensitivity and specificity appropriate for the therapeutic application of such drug conjugates, and a phase 1 trial to evaluate immunotoxin conjugate in colorectal tumors is due to commence shortly.

Therefore 791T/36 has a potential diagnostic role in the evaluation of patients with ovarian, cervical and corpus tumors and appears to offer a promising means for targeting cytotoxic drugs. The major problem still to be overcome in both these approaches to cancer diagnosis and treatment will be the development of methods to minimize the patient antimouse monoclonal antibody response, a potential drawback of these techniques.

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REFERENCES

1. Kohler G, Milstein C. Continuous cultures of fused cells, secreting antibody of predefined specificity. Nature 1975; 256:495.
2. Farrands PA, Perkins AC, Pimm MV, et al. Radioimmunodetection of human colorectal cancers by an anti-tumor monoclonal antibody. Lancet 1982;1:397-400.
3. Farrands PA, Perkins AC, Sully L, et al. Localization of human osteosarcoma by anti-tumor monoclonal antibody 791T/36. J Bone Joint Surg [Br] 1983;658:638-40.
4. Armitage NC, Perkins AC, Pimm MV, Farrands PA, Baldwin RW, Hardcastle JD. The localization of an anti-tumour monoclonal antibody (791T/36) in gastrointestinal tumours. Br J Surg 1984;71:407-12.
5. Symonds EM, Perkins AC, Pimm MV, Baldwin RW, Hardy JG, Williams DA. Clinical implications for immunoscintigraphy in patients with ovarian malignancy: a preliminary study using monoclonal antibody 791T/36. Br J Obstet Gynaecol 1985;92:270-6.
6. Perkins AC, Whalley DR, Hardy JG. Physical approach for the reduction of dual radionuclide image subtraction artefacts in immunoscintigraphy. Nucl Med Commun 1984;5:501-12.
7. Perkins AC, Pimm MV. Differences in tumour and normal tissue concentrations of iodine- and indium-labelled monoclonal antibody. 1. The effect on image contrast in clinical studies. Eur J Nucl Med 1985;11:295-9.
8. Powell MC, Worthington BS, Symonds EM. The application of MRI in gynaecology. Br J Hosp Med 1986;6:393-403.
9. Epenetos AA, Britton KE, Mather S, et al. Targeting of iodine-123–labelled tumour associated monoclonal antibodies to ovarian breast and gastrointestinal tumours. Lancet 1982:2;999-1004.
10. Granowska M, Shepard J, Britton KE, et al. Ovarian cancer: diagnosis using ^{123}I monoclonal antibody in comparison with surgical findings. Nucl Med Commun 1984;5: 485-99.
11. Deland FE, Kim EE, Goldenberg DH. In vivo radioimmunodetection of cancer. In: Wilson OG, Hardy JG, Frier M, Davis SS, eds. Radionuclide imaging of drug research. London: Croom Helm, 1982:182-202.
12. Creasman WT, Abu-Ghazaleh S, Schmidt HJ. Retroperitoneal metastatic spread of ovarian cancer. Gynecol Oncol 1978;6:447-50.
13. Luesly DM, Chan KK, Fielding JWL. Second look laparotomy in the management of epithelial ovarian carcinoma. An evaluation of 50 cases. Obstet Gynecol 1984; 64:421-6.
14. Pimm MV, Perkins AC, Armitage NC, Baldwin RW. The characteristics of blood-borne radiolabels and the effect of anti-mouse IgG antibodies on localization of radiolabelled monoclonal antibody in cancer patients. J Nucl Med 1985;26:1011-23.
15. Epenetos AA. Clinical results with regional antibody guided irradiation. Cancer Drug Deliv 1985;2:233.
16. Embleton MJ, Byers VS, Lee HM, Scannon P, Blackhall NW, Baldwin RW. Sensitivity and selectivity of ricin toxin A chain monoclonal antibody 791T/36 conjugates against human tumour cell lines. Cancer Res (in press).

Cigarette smoking and the risk of female reproductive cancer

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An epidemiologic study was conducted to evaluate the relationship between cigarette smoking and the risk of breast, endometrial, and ovarian cancers. Results indicated a clear reduction of risk for endometrial cancer among women aged 50 years and older who smoke cigarettes. Risks were significantly reduced among moderate smokers (odds ratio = 0.6), heavy smokers (odds ratio = 0.4), and former smokers (odds ratio = 0.6). No association was observed among women under age 50 years. When the relationship between cigarette smoking and breast cancer was investigated, no statistically significant association between cigarette smoking and the risk of breast cancer was observed except among women >50 years who were light smokers only. There was also a nonstatistically significant increase in risk among younger women smoking more than two packs of cigarettes a day (odds ratio = 2.0), but overall there was no evidence of any relationship. Similarly, no association between ovarian cancer and cigarette smoking was apparent, although there was a nonsignificant increase in risk among women under age 50 years who smoked 40 or more cigarettes a day or were exsmokers. (AM J OBSTET GYNECOL 1987;157:35-40.)

Key words: Endometrial cancer, smoking, ovarian cancer, breast cancer

Cigarette smoking has been associated with an increased risk of cancers at various sites in women, as it has been in men. However, among women there has been additional discussion of a possible protective effect of cigarette smoking in the development of female reproductive cancers. Cigarette smoking may reduce the age of natural menopause in some women.[1] Not all studies have reported such a lowering, however.[2] In addition, lower levels of urinary estrogens have been reported in cigarette smokers compared with nonsmokers.[3] These observations have led epidemiologists to study the relationship between cigarette smoking and three cancers thought to be estrogen related, specifically cancers of the endometrium, ovary, and breast.

Although the existing data for breast cancer are inconsistent, most investigators have found either no association between cigarette smoking and breast cancer or a slight decrease in risk among cigarette smokers.[2, 4-9] One recent investigation involving a breast cancer screening population[10] reported an increased risk of breast cancer among premenopausal women who smoked, whereas no association was observed among postmenopausal women.

The data suggesting an association between cigarette smoking and endometrial cancer are more consistent. Three recent studies in the United States have suggested a decreased risk of endometrial cancer among cigarette smokers,[7, 11, 12] although one study of deaths caused by endometrial cancer found no association with cigarette smoking.[13] Much less attention has been paid to possible relationships between ovarian cancer and cigarette use. Doll et al.[14] reported a significant increase in the risk of ovarian cancer among British female physicians compared with nonsmoking physicians. No similar excess has yet been reported among American cohorts.[15]

Material and methods

The current study was designed to assess the impact of cigarette smoking on the risk of breast, endometrial, and ovarian cancers. An epidemiologic case-control study was conducted of all incident cases diagnosed in 1981 among Florida residents. Cases consisted of women with breast cancer (ICD-0 174), endometrial cancer (ICD-0 182), and ovarian cancer (ICD-0 183). Control subjects consisted of all female residents of the state first diagnosed in 1981 with either colon cancer (ICD-0 153), rectal cancer (ICD-0 154), cutaneous melanoma (ICD-0 173, morphology 872-878), or endocrine neoplasms (ICD-0 194-4). Control sites were selected on the basis of an absence of a recognized association with cigarette smoking.

The study population was identified through the records of the Florida Cancer Data System. This statewide cancer registry collects information on all cases of cancer diagnosed in state-licensed hospitals in Florida. In addition to relevant hospitalization and tumor information, the Florida Cancer Data System collects data

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Table I. Characteristics of subjects with cancer and control subjects

Characteristic	*Breast cancer cases*		*Endometrial cancer cases*		*Ovarian cancer cases*		*Controls*	
	No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
Age (yr)								
<50	838	16	113	8	138	16	364	9
50-64	1624	31	492	36	285	32	773	20
65-74	1576	30	500	36	267	30	1284	33
≥75	1208	23	269	20	199	22	1500	38
Race								
White	4853	93	1281	93	838	94	3726	95
Nonwhite	365	7	87	7	49	6	184	5
Unknown	28	0.5	6	0.4	2	0.2	10	0.3
Marital status								
Single	290	6	71	5	79	9	232	6
Married	3107	59	801	58	493	55	1914	49
Divorced/widowed	1759	34	472	34	311	35	1723	44
Unknown	90	1	30	3	6	1	52	1
Smoking habits								
Never smoked	2617	50	749	55	471	53	2111	54
<20 cigarettes/day	471	9	93	7	69	8	248	6
20-40 cigarettes/day	398	8	59	4	66	7	212	5
>40 cigarettes/day	116	2	12	1	17	2	60	2
Exsmoker	392	7	71	5	67	8	314	8
Other tobacco	17	0.3	6	0.4	6	0.7	7	0.2
Unknown	1235	24	384	28	193	21	969	25
Total cases	5246	100	1374	100	889	100	3921	100

on the birth date, race, sex, marital status, and smoking characteristics of all reported cases. Tobacco use is classified by type of product (cigarette, cigar, pipe, or smokeless tobacco) and, for current cigarette smokers, packs of cigarettes smoked per day. For former smokers, no information on quantities previously smoked or the elapsed smoke-free interval is available.

The measure of association between cigarette smoking and the risk of cancer used was the relative risk as estimated by the odds radio. To assess the cancer risks associated with cigarette smoking among younger and older women, odds ratios were calculated separately for those participants <50 years of age and those aged 50 years and older as an approximate measure of menopause status. Because the control population was considerably older that the case population, age-adjusted estimates were calculated according to the method developed by Woolf.[16] Finally a series of multiple logistic regression models was used to calculate odds ratios adjusted for age, race, tobacco use, and marital status. To adjust for the high percentage of unknowns in the smoking category, the category "smoking status unknown" was included in the logistic regression models.

Results

There were 5246 women with breast cancer, 1374 women with endometrial cancer, and 889 women with ovarian cancer included as cases in this study. Control subjects consisted of 2416 women with colon cancer, 899 with rectal cancer, 420 with cutaneous melanoma, and 186 with endocrine neoplasms. When case and control subjects were compared in terms of demographic characteristics (Table 1), the control subjects tended to be older than the patients with either breast, endometrial, or ovarian cancer. There was little difference in the composition of the groups by race or marital status. There was a tendency for a higher proportion of control women to report their marital status as widowed or divorced. The variation in marital status primarily reflected the higher proportion of control women in the oldest age groups. When the smoking characteristics of the study population were considered, 50% of the patients with breast cancer, 55% of the patients with endometrial cancer, 53% of the patients with ovarian cancer, and 54% of control women were nonsmokers.

Breast cancer. When the relationship between cigarette smoking and the risk of development of breast cancer was considered, current cigarette smokers had a 50% greater risk of developing breast cancer than did nonsmokers (Table II). When the number of cigarettes smoked per day was considered, there was a significant excess risk at each smoking level but little evidence of any dose-response trend. Women smoking less than two packs of cigarettes a day had a 50% increase in risk, which increased to 60% among women smoking more than two packs a day. No excess risk was observed among former smokers.

Because the relationship between the risk of breast cancer and cigarette smoking may vary by menopausal status, breast cancer cases were subdivided into women aged <50 years at diagnosis and women aged 50 years

Table II. Relationship between cigarette smoking and female reproductive cancers: unadjusted odds ratios and confidence intervals

Age group	*Smoking category*	*Breast cancer*	*Endometrial cancer*	*Ovarian cancer*
All ages	<20 cigarettes/day	1.5 (1.3, 1.8)	1.1 (0.8, 1.4)	1.2 (0.9, 1.7)
	20-40 cigarettes/day	1.5 (1.3, 1.8)	0.8 (0.6, 1.1)	1.4 (1.0, 1.9)
	>40 cigarettes/day	1.6 (1.1, 2.1)	0.6 (0.3, 1.0)	1.3 (0.7, 2.2)
	Exsmoker	1.0 (0.9, 1.2)	0.6 (0.5, 0.8)	1.0 (0.7, 1.3)
Before age 50 yr	<20 cigarettes/day	1.2 (0.8, 1.8)	1.0 (0.5, 1.9)	0.5 (0.2, 1.1)
	20-40 cigarettes/day	1.4 (0.8, 2.0)	0.9 (0.4, 1.8)	1.0 (0.5, 2.0)
	>40 cigarettes/day	2.3 (0.9, 6.2)	1.9 (0.4, 8.4)	2.8 (0.8, 10.0)
	Exsmoker	1.3 (0.8, 2.2)	0.8 (0.3, 2.2)	2.0 (0.9, 4.1)
Aged 50 yr and older	<20 cigarettes/day	1.5 (1.3, 1.9)	1.1 (0.8, 1.4)	1.4 (1.1, 1.9)
	20-40 cigarettes/day	2.0 (1.6, 2.4)	0.8 (0.6, 1.1)	1.6 (1.2, 2.2)
	>40 cigarettes/day	1.8 (1.3, 2.5)	0.5 (0.2, 0.9)	1.0 (0.6, 2.0)
	Exsmoker	1.1 (1.0, 1.3)	0.6 (0.5, 0.8)	0.9 (0.6, 1.2)

Table III. Relationship between cigarette smoking and female reproductive cancers: age–adjusted odds ratios and confidence intervals

Age group	*Smoking category*	*Breast cancer*	*Endometrial cancer*	*Ovarian cancer*
All ages	<20 cigarettes/day	1.3 (1.1, 1.5)	0.9 (0.7, 1.1)	1.1 (0.8, 1.5)
	20-40 cigarettes/day	1.2 (1.0, 1.4)	0.7 (0.5, 0.9)	1.1 (0.8, 1.5)
	> 40 cigarettes/day	1.2 (0.8, 1.6)	0.6 (0.3, 1.0)	1.1 (0.6, 1.9)
	Exsmoker	0.9 (0.8, 1.0)	0.6 (0.4, 0.8)	0.9 (0.7, 1.2)
Before age 50 yr	<20 cigarettes/day	1.2 (0.8, 1.8)	0.9 (0.4, 1.8)	0.4 (0.2, 1.0)
	20-40 cigarettes/day	1.3 (0.8, 1.9)	0.9 (0.4, 1.8)	1.0 (0.5, 1.9)
	>40 cigarettes/day	2.0 (0.8, 5.1)	1.9 (0.5, 7.8)	2.4 (0.8, 8.0)
	Exsmoker	0.9 (0.5, 1.6)	0.9 (0.3, 2.3)	2.0 (1.0, 4.1)
Aged 50 yr and older	<20 cigarettes/day	1.3 (1.1, 1.6)	0.9 (0.7, 1.2)	1.2 (0.9, 1.7)
	20-40 cigarettes/day	1.2 (1.0, 1.5)	0.6 (0.4, 0.9)	1.2 (0.8, 1.6)
	>40 cigarettes/day	1.1 (0.8, 1.5)	0.4 (0.2, 0.8)	0.9 (0.5, 1.6)
	Exsmoker	0.9 (0.8, 1.1)	0.6 (0.4, 0.8)	0.8 (0.6, 1.1)

or older. In younger women there were no statistically significant excess risks observed, although there was evidence of a trend to higher risks with increasing number of cigarettes smoked per day. Among younger women, risk estimates increased from 1.2 among light smokers to 2.3 among those smoking more than two packs of cigarettes per day. Former smokers continued to have no excess risk of breast cancer. Adjustment for differences in age between case and control subjects under age 50 years resulted in only a slight decrease in risk, with risks for heavy smokers decreasing from 2.3 to 2.0 (Table III).

Among women aged fifty years and older, there was a statistically significant increase in breast cancer risk among smokers compared with nonsmokers. A 50% increase in risk was observed among women smoking less than 20 cigarettes a day, with a doubling of risk observed among those smoking 20 to 40 cigarettes a day. Among the heaviest smokers, an 80% increase in risk was observed. This risk appears slightly lower than that of the previous smoking category but is based on a small number of women and is not significantly different from the previous estimate. However, adjustment for age had a major impact on these risk estimates, with risks decreasing to close to unity for each smoking level, although the 30% increase in risk of light smokers remained statistically significant.

Endometrial cancer. When the relationship between endometrial cancer and cigarette smoking was considered, there was a clear indication of a decreased risk with increasing number of cigarettes smoked per day. The odds ratios decreased from 1.1 among light smokers to 0.6 among heavy smokers. However, the risk reduction was not statistically significant except for former smokers. When the patients with endometrial cancer were subdivided by age, different trends emerged for younger and older women. Among patients with endometrial cancer diagnosed before age 50 years, there was no evidence of an association between cigarette smoking characteristics and risk of endometrial cancer either before or after age adjustment of the risk estimates. Among women aged fifty years and older there was a clear trend of decreasing risk with increasing number of cigarettes smoked per day. Women smoking more than two packs of cigarettes a day had only 40% of the risk of endometrial cancer of nonsmoking women whereas those smoking 20 to 40 cigarettes a day had 60% of the risk of nonsmokers. Women who stopped smoking appeared to retain their lower endometrial cancer risk status.

Table IV. Odds ratios for tobacco use and reproductive cancers adjusted for age, race, marital status, and tobacco use

Tobacco use	*Breast cancer*	*Endometrial cancer*	*Ovarian cancer*
<20 cigarettes/day	1.3 (1.1, 1.5)	0.9 (0.7, 1.2)	1.1 (0.8, 1.4)
20-40 cigarettes/day	1.2 (1.0, 1.5)	0.7 (0.5, 0.9)	1.2 (0.8, 1.6)
>40 cigarettes/day	1.3 (1.0, 1.8)	0.5 (0.3, 0.9)	1.1 (0.6, 1.9)
Exsmoker	1.0 (0.8, 1.1)	0.6 (0.5, 0.8)	0.9 (0.7, 1.2)

Table V. Adjusted odds ratios for tobacco use and breast cancer by age group

Cigarette smoking	*Before age 50 yr*	*Age 50 yr and older*
<20 cigarettes/day	1.2 (0.8, 1.8)	1.3 (1.0, 1.5)
20-40 cigarettes/day	1.3 (0.8, 2.0)	1.1 (0.9, 1.4)
>40 cigarettes/day	2.1 (0.8, 5.8)	1.1 (0.8, 1.5)
Exsmoker	1.2 (0.7, 2.0)	0.9 (0.7, 1.0)

Ovarian cancer. Among patients with ovarian cancer, no relationship of risk to cigarette smoking characteristics could be observed. Among younger women there was some suggestion of an increased risk with increasing number of cigarettes smoked. Odds ratios increased from 0.5 among light smokers to > 2.0 among women smoking more than two packs of cigarettes a day. However, the results were not statistically significant either before or after adjustment for age. Among older women, no clear pattern emerged. After adjustment for age there were no excess risks associated with any level of cigarette smoking.

A logistic regression analysis was used to assess the relationship between each of these cancers and cigarette smoking after adjustment for differences in age, race, and marital status and those with unknown cigarette use. Table IV indicates the tobacco–related adjusted odds ratios for breast, endometrial, and ovarian cancers. The excess risk of breast cancer among cigarette smokers was reduced substantially after adjustment, with no remaining evidence of a dose-response trend or statistically significant elevations in risk except among light smokers. Because it appeared that the adjustment process might be masking the risk among the younger but smaller number of patients with breast cancer, separate logistic models were developed for women under and over 50 years of age (Table V). For older women, results remained essentially identical to the combined analysis. Among younger women the adjusted risks increased from 1.2 in light smokers to 2.1 in heavy smokers, almost identical to the age only–adjusted risks, and remained not statistically significant. Among older women the excess risk associated with smoking 20 cigarettes a day or less was not statistically significant after adjustment for these additional factors.

When the relationship between endometrial cancer and cigarette smoking was considered, a clear pattern of decreased risk with increasing use of cigarettes emerged. Smokers of a pack or more of cigarettes a day experienced a statistically significant reduction in risk of endometrial cancer compared with nonsmokers. The reduction in risk increased with the number of cigarettes smoked per day. There was a 50% decrease in the risk of endometrial cancer in the women smoking more than two packs of cigarettes a day. Women smoking between one and two packs a day had a 30% decrease in risk, whereas smokers of less than one pack a day had a slight (10%) but not statistically significant decrease in risk. Former smokers had a 40% decrease in risk compared with nonsmokers. For ovarian cancer, no evidence of any association between the use of cigarettes and cancer risk was observed. Odds ratios approximated 1.0 at all smoking levels, indicating no association between cigarette use and risk of ovarian cancer.

Comment

This study confirms the observation of several other authors of a decreased risk of endometrial cancer in women who smoke cigarettes.[7, 11, 12] Results of the current study indicate that the decreased risk is limited to women aged 50 years and over. Among these women the risk of endometrial cancer decreased in a dose-response fashion as the number of cigarettes smoked per day increases. Women smoking less than 20 cigarettes a day have no significant decrease in risk. Women smoking one to two packs a day have an estimated 40% decrease in risk. Among heavier smokers, a 60% decrease in risk of endometrial cancer is observed. Former smokers appear to retain a significant decrease in risk of endometrial cancer, having only 60% of the risk of nonsmokers. With no information available on length of smoking cessation or previous smoking habits of former smokers, it is not possible to determine whether former smokers retain their preferential risk status for long periods of time. Among women <50 years of age, no decreased risk of endometrial cancer associated with cigarette smoking was observed.

The study also evaluated the relationship between cigarette smoking and the risk of ovarian and breast cancer. Analyses of the data for these two sites revealed no significant association between cigarette smoking and the risk of either ovarian or breast cancer. Among younger women an increase in risk of breast cancer among cigarette smokers, particularly smokers of more than 40 cigarettes a day, was observed but was not statistically significant. Schechter et al.[10] have reported a significant increase in breast cancer in premenopausal women who smoke cigarettes attending a breast cancer screening program. In England, Vessey et al.[5] similarly noted a low risk of breast cancer among cigarette smokers. However, two recent investigations[2, 8] found no association between cigarette smoking and breast cancer risk in either premenopausal or postmenopausal women.

Noting lower levels of urinary estrogens in cigarette smokers compared with nonsmokers, MacMahon et al.[3] suggested that smokers may have a lower risk of breast cancer than nonsmokers. The current study found no evidence of a decreased risk of breast cancer among cigarette smokers.

No association was observed between cigarette smoking and the risk of ovarian cancer among women of any age. If cigarette smoking has an "antiestrogenic" effect, no impact on the risk of ovarian cancer can be observed.

In considering the findings of this study, several methodologic issues must be addressed. In the current investigation the smoking habits of women with breast, endometrial, and ovarian cancer were compared with women with other malignancies. If the smoking habits of women with cancers of these other sites do not reflect the smoking characteristics of the general population, bias in the calculation of the risk estimates could have occurred. Some support for the findings of this study could come from studies with similar findings that had used community control subjects. However, community control subjects have been used only rarely. The findings of the current study with regard to breast cancer are consistent with those of Lesko et al.,[11] Smith et al.,[7] and Rosenberg et al.[8] All three studies used women with other malignancies as their control populations. Two studies that used community control subjects reported decreases in risk among cigarette smokers similar to those of the current study. In a study of endometrial cancer among women aged 55 years and under with community control subjects, Tyler et al.[17] observed a 30% decrease in risk of endometrial cancer among women aged 50 to 54 years who smoke cigarettes. Weiss et al.[12] reported a 60% decrease in risk in women who had ever smoked after adjustment for age, parity, weight, hypertension, and estrogen use. Results of both studies are consistent with the decreased risk of endometrial cancer among cigarette smoking observed in the current study.

The study population was divided into those <50 and those >50 years of age as an approximate measure of menopause status. The median age of menopause for American women appears to be around age 52 years.[1, 2] The selection of any specific age as a cutoff point is somewhat arbitrary when used as a surrogate measure for menopause status that does not occur at the same age in all women. However, calculation of age–specific odds ratios for women under 45, 45 to 49, 50 to 54, and 55 years and older indicated that the risks experiences by women aged 45 to 49 more closely approximated those of younger women whereas the risks of those aged 50 to 54 years resembled those of women aged 55 years and over, suggesting that age 50 years was the best cutoff point in the absence of data on menopause status.

Use of the statewide population–based Florida Cancer Data System permitted the study of a large number of cancer cases. However, the disadvantage of the registry data set was the lack of information pertaining to risk factors such as obesity, parity, and exogenous estrogen use. Obesity was of particular concern as a possible confounding factor because cigarette smokers may weigh less than nonsmokers.[1] Information of tobacco use was missing for approximately 25% of case and control subjects. To attempt to minimize any bias associated with this lack of data, the logistic regression procedures adjusted to lack of tobacco data. Despite the lack of these data, the findings of the current study are in agreement with those of studies able to consider these additional risk factors.[7, 17]

The reduction in risk of endometrial cancer in postmenopausal women who smoke cigarettes has now been reported by a number of epidemiologic investigations. The current study has provided additional support for the causative nature of the relationship through documentation of a clear dose-response relationship. Further elucidation of the mechanism by which cigarette smoking affects this reduction in risk is required. However, from a public health perspective the relatively small reduction in the incidence of endometrial cancer attributable to cigarette smoking is far exceeded by the increase in incidence of other cancers in women who smoke cigarettes.

REFERENCES

1. Willett W, Stapfer MJ, Bain C, et al. Cigarette smoking, relative weight and menopause. Am J Epidemiol 1983;117:651-8.
2. Brinton LA, Schairer C, Stanford JL, Hoover RN. Cigarette smoking and breast cancer. Am J Epidemiol 1986; 123:614-22.
3. MacMahon B, Trichopoulos D, Cole P, Brown J. Cigarette smoking and urinary estrogens. N Engl J Med 1982; 307:1062-5.
4. Hiatt RA, Fireman BH. Smoking, menopause, and breast cancer. JNCI 1986;76:833-8.
5. Vessey M, Baron J, Doll R, McPherson K, Yeates D. Oral contraceptives and breast cancer: final report of an epidemiologic study. Br J Cancer 1983;47:455-62.
6. Kelsey JL, Fischer DB, Holford TR, et al. Exogenous es-

trogens and other factors in the epidemiology of breast cancer. JNCI 1981;67:327-33.
7. Smith EM, Sowers MF, Burns TL. Effects of smoking on the development of female reproductive cancers. JNCI 1984;73:371-6.
8. Rosenberg L, Schwingl PJ, Kaufman DW, et al. Breast cancer and cigarette smoking. N Engl J Med 1984;310: 92-4.
9. Hiatt RA, Friedman OD, Bawol RD, Ury KH. Breast cancer and serum cholesterol. JNCI 1982;68:885-9.
10. Schechter MT, Miller AB, Howe GR. Cigarette smoking and breast cancer: a case-control study of screening program participants. Am J Epidemiol 1985;121:479-87.
11. Lesko SM, Rosenberg L, Kaufman DW, et al. Cigarette smoking and the risk of endometrial cancer. N Engl J Med 1985;313:593-6.
12. Weiss NS, Farewell VT, Szekely DR. Estrogens and endometrial cancer: effects of other risk factors on the association. Maturitas 1980;2:185-90.
13. Garfinkel L. Cancer mortality in nonsmokers: prospective study of the American Cancer Society. JNCI 1980; 65:1169-73.
14. Doll R, Gray R, Hafner B, Peto R. Mortality in relation to smoking: twenty-two years' observations on female British doctors. Br Med J 1980;280:967-71.
15. Hammond EC. Smoking in relation to death rates of one million men and women. Natl Cancer Inst Monogr 1966;19:127-204.
16. Woolf B. On estimating the relation between blood group and disease. Ann Hum Genet 1955;19:251-3.
17. Tyler OW Jr, Webster LA, Ory HW, Rubin GL. Endometrial cancer: how does cigarette smoking influence the risk of women under age fifty-five years having this tumor? AM J OBSTET GYNECOL 1985;151:899-905.

Uterine and umbilical artery velocimetry during normal labor

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Twelve normal parturients were studied with a continuous wave Doppler unit to assess changes in uterine and umbilical velocity waveforms during labor. The analysis of these waveforms included the peak systolic/end-diastolic ratio and the evaluation of a diastolic notch. Each woman served as her own control, and all fetal heart rate tracings were normal. In latent phase labor and intact membranes, the umbilical artery systolic/end diastolic ratios before, during, and after a contraction were 2 ± 0.2, 2 ± 0.3 and 1.95 ± 0.3 (N.S.). Similar results were obtained in the active phase, after rupture of membranes, or during oxytocin stimulation. This stability of the fetal cardiovascular system ensures an uninterrupted gas exchange process during the contractions (on the fetal side), enabling the great majority of term fetuses to tolerate labor with minimal if any metabolic changes. The uterine artery end-diastolic velocity fell progressively during the contraction, reaching 0 when the intrauterine pressure exceeded 35 mm Hg. Despite intrauterine pressure of >60 mm Hg, the diastolic notch did not appear. Thus at term, the umbilical artery velocity waveform does not change over a wide range of uterine pressures. The changes seen in the uterine artery waveforms suggest that the end-diastolic component is primarily determined by changes in the arcuate and spiral arteries, both of which are affected during the uterine contraction. (AM J OBSTET GYNECOL 1987;157:40-3.)

Key words: Doppler, systolic/end-diastolic ratio, uterine and umbilical artery waveforms

Recent advances in Doppler technology have allowed obstetricians to study umbilical and uterine blood velocity patterns in the undisturbed human pregnancy. A progressive fall in vascular resistance as reflected by changes in the velocity waveforms has been demonstrated during normal pregnancy for both uterine and umbilical circulations.[1-4] The acute changes that may take place during labor or after rupture of membranes, however, are less well understood. These changes may be of importance in the understanding of fetal distress in labor and further our understanding of fetal adaptation to stress.

Material and methods

The patient population consisted of 12 pregnant women, at term, admitted to labor and delivery in latent phase labor with intact membranes. All had an uncom-

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plicated antepartum course, and the estimated fetal weight was considered to be average for gestational age. Eight patients were multiparous and four were primiparous. With the patient in the lateral decubitus position, the umbilical velocity waveforms were obtained by placing the transducer in the periumbilical area and changing the angle until a typical umbilical artery waveform (Fig. 1) was obtained. The equipment and methodology used have been described in detail previously.[5] In short, it consists of a continuous wave Doppler unit with a power density of 6.5 mW/cm_2 and a 4 mHz transducer.

The Doppler shift is analyzed by a spectrum analyzer with Fast Fourier Transform. The waveforms obtained display the frequency vertically and time horizontally.

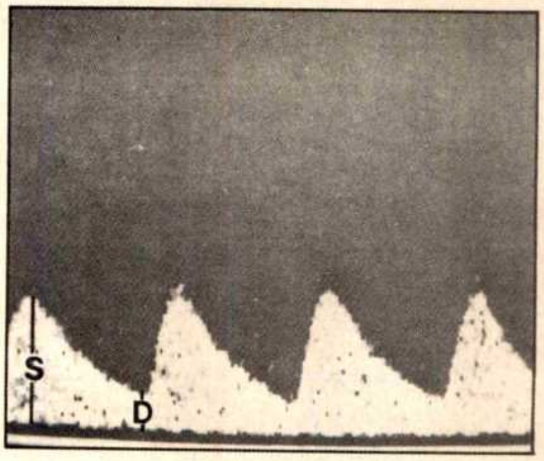
Since Doppler shift is proportional to the velocity of the reflectant object, these waveforms represent the changes of blood velocity during the cardiac cycle. Continuous recordings of these velocity waveforms are then stored on a JVC audio unit.

During the initial study,[5] the confirmation of the umbilical artery was obtained by real-time imaging, but it soon became apparent that the umbilical artery waveforms could be reliably obtained without the use of imaging devices. The measurements presented in this report were obtained without the use of such devices. These characteristic waveforms were then recorded before, during, and after the contractions.

The uterine artery waveforms were also obtained transabdominally by placing the Doppler probe in the right or left lower quadrant, aimed toward the lower uterine segment and moving it slowly until the appropriate waveforms are seen. Once the typical arterial waveforms were obtained, they were recorded on a JVC audio unit before, during, and after the contraction without changing the position or angle of the transducer. The onset, peak, and end of the contraction, as well as the intensity of the contractions (assessed every 10 seconds), were appropriately marked on the tape.

These arterial waveforms were recorded during latent phase, active phase, and second stage of labor. Similar measurements were also taken before and after membranes ruptured. In five patients, the membranes ruptured spontaneously, whereas the other seven had artificial rupture of membranes. Four of the 12 patients had an arrest of labor and required oxytocin stimulation. All four patients had intrauterine pressure catheters placed before the infusion of oxytocin. For these patients, the measurements were also done immediately before and during the infusion of oxytocin.

The analysis of the waveforms consisted of the calculation of systolic/end-diastolic ratio and the presence or absence of a diastolic notch. The systolic/end-diastolic ratio used in the analysis represents the average measure of four different waveforms. The analysis was performed only on intervals in which the fetal heart rate stayed between 120 and 160 bpm. We used these numbers because of our previous observation that within 120 to 160 bpm, changes in fetal heart rate will not cause significant changes in the systolic/end-diastolic ratio.



**Signal produced by Doppler transducer.
S = Systolic, D = Diastolic**

Fig. 1. Umbilical artery velocity waveforms.

Results

All twelve women had normal vaginal deliveries. The fetal heart rate tracings were normal with the exception of occasional variable decelerations noticed during the latter part of the second stage of labor. The mean birth weight was 3200 ± 400 gm. All infants had 1- and 5-minute Apgar scores >7. None of the infants were admitted to the neonatal intensive care unit. The duration of labor (in the hospital) was 4½ hours.

Measurement of the umbilical artery systolic/end-diastolic ratios were taken during latent phase labor before, during, and after uterine contractions. Their mean ± 2 SD were 2 ± 0.25, 2 ± 0.3, and 1.95 ± 0.3, respectively. The differences were not statistically significant. Similar results were obtained for active phase and second stage labor. Table I illustrates the umbilical artery systolic/end-diastolic ratios (mean ± 2 SD) after rupture of membranes and during oxytocin stimulation. There was no difference in the systolic/end-diastolic ratios after either of these obstetric procedures. Uterine artery waveforms, however, changed during the contractions (Fig. 2).

A progressive decrease in the diastolic component occurred with an increase in intrauterine pressure. At pressures >35 mm Hg the end-diastolic velocity be-

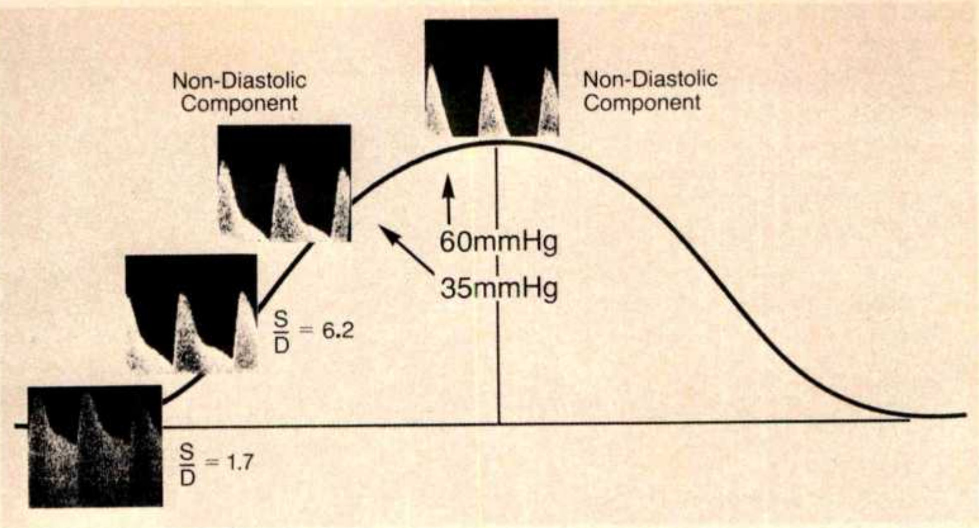


Fig. 2. Changes in uterine artery waveforms during contractions.

Table I. Umbilical artery systolic/end-diastolic ratios* after rupture of membranes or during oxytocin stimulation

	Between contractions	*During contractions*
Intact membranes	2 ± 0.25	2 ± 0.3
Ruptured membranes	1.95 ± 0.25	2 ± 0.25
Before oxytocin	2.1 ± 0.3	2 ± 0.3
During oxytocin	1.9 ± 0.2	2 ± 0.2

*($\bar{x}$ ± 2 SD).

came 0. At no time during the contraction did we note the appearance of a diastolic notch.

Comment

Our results confirmed the report by Stuart et al.[6] regarding the lack of changes of umbilical artery waveforms during labor. Furthermore, we are able to document the absence of changes in the umbilical artery velocity systolic/end-diastolic ratio throughout labor and over a wide range of intrauterine pressures. Stimulation with oxytocin or labor in the presence of rupture of membranes did not seem to change the velocity patterns. McCallum et al.[7] reported a patient in whom there was an abnormal systolic/end-diastolic ratio in the presence of premature rupture of membranes. In that patient the pregnancy ended in a stillbirth. It is therefore possible that factors other than just premature rupture of membranes were responsible for the abnormal velocity waveforms in that particular patient.

The velocity waveforms represent changes in the frequency of the reflected sound wave (vertically) as a function of time (horizontally). The frequency of any reflected sound is proportional to the speed with which the reflecting object moves. In our case the reflecting objects are the red blood cells. Therefore it is reasonable to assume that changes noted in these waveforms will reflect changes in blood velocity.

The ascending slope represents the increase in blood velocity at the beginning of systole and is determined primarily by stroke volume. The descending slope represents the fall in blood velocity during diastole and is determined primarily by vessel wall compliance and the peripheral resistance of that particular vascular bed. Throughout labor, with intact or ruptured membranes, we could find no change in the umbilical artery waveforms. One may then postulate that fetal cardiac stroke volume (as reflected by ascending slope) and placental vascular resistance (as reflected by the diastolic velocity) are remarkably stable in the term fetus despite a wide range of intrauterine pressure (10 to 60 mm Hg). This stability of the fetal cardiovascular system ensures an uninterrupted gas exhange process during the contractions (on the fetal side), which enables the great majority of term fetuses to tolerate labor with minimal if any metabolic changes.

The changes in uterine velocity waveforms during labor were of considerable interest. Before labor, the changes in uterine artery velocity waveforms, which characterize the normal pregnancy, consist of the loss of diastolic notch and a progressive decrease in the systolic/end-diastolic ratio.[8] In normal pregnancies these changes are seen as early as 16 weeks' gestation, are completely developed at 26 weeks, and are relatively unchanged for the remainder of the pregnancy. We postulate that these changes in the velocity waveforms reflect the improvement in arterial wall compliance and the development of the uteroplacental vascular bed. During the uterine contractions, the spiral arteries are being constricted in proportion to the intensity of the contraction. The impact of these changes on the uterine artery waveforms is a progressive fall of the diastolic velocity. The end-diastolic velocity reaches 0 when intrauterine pressure exceeds 35 mm Hg. Despite pressures as high as 60 mm Hg, no diastolic notch was noticed. This suggests that the end-diastolic component is primarily determined by changes in the arcuate and spiral arteries, both of which are affected during the uterine contraction.

In contrast to these changes, pregnancies complicated by severe preeclampsia, for example, will have abnormal uterine artery waveforms characterized by a high systolic/end-diastolic ratio and a distinct diastolic notch, similar to that seen early in pregnancy or in the nonpregnant state.[4] The genesis of this abnormal pattern during the third trimester of pregnancy is not well understood. However, inferring from the changes just described during labor, one may postulate that the mechanism involved includes not only peripheral vasoconstriction (reflected by a high systolic/end-diastolic ratio) but also lack of development of uterine artery compliance.

The following conclusion can be made: (1) The umbilical artery velocity waveforms seen in the term average for gestational age infant do not change during labor. This status quo does not seem to be affected by the stage of labor, status of membranes, or oxytocin stimulation. (2) The uterine artery velocity waveforms do change during labor. The change consists of a progressive fall in the diastolic velocity, the degree of which is related to the intensity of the uterine contractions. With intrauterine pressures >35 mm Hg, no forward end-diastolic flow is noticed.

REFERENCES

1. FitzGerald DE, Drumm JE. Non-invasive measurement of human fetal circulation using ultrasound: a new method. Br Med J 1977;2:1450.
2. Stuart B, Drumm J, FitzGerald DE, Duignan NM. Fetal blood velocity waveforms in normal pregnancy. Br J Obstet Gynaecol 1980;87:780.
3. Fleischer A, Schulman H, Farmakides G, Bracero L, Blattner P, Randolph G. Umbilical artery velocity waveforms and intrauterine growth retardation. AM J OBSTET GYNECOL 1985;151:502.
4. Fleischer A, Schulman H, Farmakides G, et al. Uterine artery Doppler velocimetry in pregnant women with hypertension. AM J OBSTET GYNECOL 1986;154:806.
5. Schulman H, Fleischer A, Stern W, Farmakides G, Jagani N, Blattner P. Umbilical velocity wave ratios in human pregnancy. AM J OBSTET GYNECOL 1984;148:985.
6. Stuart B, Drumm J, FitzGerald DE, Duignan NM. Fetal blood velocity waveforms in uncomplicated labour. Br J Obstet Gynaecol 1981;88:865.
7. McCallum WD, Williams CS, Napel S, Daigle RE. Fetal blood velocity waveforms. AM J OBSTET GYNECOL 1978; 132:425.
8. Schulman H, Fleischer A, Farmakides G, Bracero L, Rochelson B, Grunfeld L. The development of uterine artery compliance in pregnancy as detected by Doppler ultrasound. AM J OBSTET GYNECOL (In press.)

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Percutaneous ultrasound-guided fetal blood sampling in the management of nonimmune hydrops fetalis

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Nonimmune hydrops fetalis can be caused by fetal chromosomal, hematologic, cardiac, or infectious conditions. A fetal blood sample obtained from percutaneous ultrasound-guided fetal blood sampling can offer vital information about the underlying cause of nonimmune hydrops fetalis, and perinatal management can be planned accordingly. Ten cases of nonimmune hydrops fetalis were investigated with percutaneous ultrasound-guided fetal blood sampling and subsequent fetal blood analysis. Nine were proved to be Bart's hemoglobin hydrops fetalis, with 78.2% to 99% of Bart's hemoglobin in the fetal blood. Terminations of pregnancy were undertaken immediately, resulting in a lowered maternal morbidity rate. Free cord loops or an intra-abdominal portion of the dilated umbilical vein can be used for percutaneous fetal blood sampling in nonimmune hydrops fetalis when the placental cord insertion is difficult to visualize. Percutaneous ultrasound-guided fetal blood sampling and subsequent fetal blood analysis will eventually play a key role in the management of the puzzling nonimmune hydrops fetalis if the safety of ultrasound-guided fetal blood sampling can be verified by further clinical studies. (AM J OBSTET GYNECOL 1987;157:44-9.)

Key words: Nonimmune hydrops fetalis, ultrasound-guided fetal blood sampling, α-thalassemia, Bart's hemoglobin hydrops fetalis

Nonimmune hydrops fetalis carries substantially high perinatal mortality and maternal morbidity rates.[1] Etiologically it is a heterogeneous group. Chromosomal, hematologic, cardiac, and infectious causes have all been described.[2, 3] The diagnosis of nonimmune hydrops fetalis is not difficult. However, to define its underlying cause is not an easy task. Although ultrasound is helpful in conditions like fetal cardiac malformation or arrhythmia, it alone cannot reliably predict prognosis and survival.[4] A fetal blood sample obtained under ultrasound guidance can be of enormous help in elucidating the underlying cause of nonimmune hydrops fetalis and in guiding the ensuing management, since it can provide critical clues about the chromosomal, hematologic, and infectious states of the fetus.

α-Thalassemia accounts for 56.7% of nonimmune hydrops fetalis in Taiwan.[5] Even in the United States and other parts of the world, it is increasingly seen as a cause of nonimmune hydrops fetalis accompanying an influx of Southeast Asian immigrants.[6, 7]

Fetal blood sampling along with simple hemoglobin electrophoresis will provide rapid diagnosis of this hopeless fetal condition, and the ensuing early termination could minimize the high risk of maternal complications through careful obstetric management. Since nonimmune hydrops fetalis of other etiology may not be fatal once the lethal Bart's hemoglobin hydrops fetalis is excluded by fetal blood electrophoresis, aggressive perinatal management can be instituted by exploiting information yielded from further analysis of the fetal blood sample.

Since July 1985 we have added ultrasound-guided fetal blood sampling to our management protocol for nonimmune hydrops fetalis. We report our 1-year experience herein.

Material and methods

From July 1985 to June 1986, 10 pregnant women were referred to National Taiwan University Hospital for consultation because of previous or present hydrops fetalis. Gestational age ranged from 21 to 35 weeks (mean, 29.7 weeks). All mothers were Rh D positive, with four of them having had a previous hydropic birth. On ultrasound examination all the fetuses were frankly hydropic except one case (case 1) (Table I). Careful ultrasound scanning revealed no other evident malformation. Special attention was paid to the fetal heart structure and rhythm and the presence of placental tumor. Oligohydramnios and bulky placenta were noted except in cases 1 and 2.

Since α-thalassemia was the most frequent cause of nonimmune hydrops fetalis in this geographic region,

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Table I. Age, gestational week, history, and ultrasound findings in all cases in this series

Case	Age (yr)	Gestational age (wk)	Previous hydrops	*Ultrasound finding* Fetal edema	Placenta	Amniotic fluid
1	29	21	Yes (etiology?)	No	Normal thickness, previa	Polyhydramnios
2	27	31	Yes (etiology?)	Frank hydrops	Bulky	Normal amount
3	27	35	No	Frank hydrops	Bulky	Oligohydramnios
4	28	35	Yes (etiology?)	Frank hydrops	Bulky	Oligohydramnios
5	23	30	No	Frank hydrops	Bulky	Oligohydramnios
6	26	32	No	Frank hydrops	Bulky	Oligohydramnios
7	23	25	No	Frank hydrops	Bulky	Oligohydramnios
8	35	25	Yes (etiology?)	Frank hydrops	Bulky	Oligohydramnios
9	31	31	No	Frank hydrops	Bulky	Oligohydramnios
10	30	32	No	Frank hydrops	Bulky	Normal amount

ultrasound-guided fetal blood sampling for fetal blood hemoglobin electrophoresis was suggested as the initial step of management. After the parents were counseled according to the existing information about ultrasound-guided fetal blood sampling, all couples elected to have the procedure.

After abdominal skin was twice sterilized with povidone-iodine-alcohol solution, the placental cord insertion was identified by careful scanning with a sector scanner (Model SSD-280, 3.5 MHz, long focus, Aloka Co., Tokyo, Japan). The needle guide was attached at a 45-degree angle to the sector probe. A puncture line was generated on the monitor screen to indicate the needle path. After a proper cord segment adjacent to the placental cord insertion was selected, a puncture line was made crossing the target. A 22-gauge needle with stylet was rapidly advanced through the needle guide to penetrate maternal skin, uterine wall, sometimes placenta, and amniotic cavity, until it reached the margin of the cord. The venous wall was then penetrated with a short thrust of the needle, and the echo of needle tip could be seen lying within the lumen. Three to ten milliliters of fetal blood was then aspirated with a disposable syringe without anticoagulant. The blood obtained was rapidly dispensed to adequate containers for various analyses. In later cases in this series mean corpuscular volume of the aspirated blood was checked immediately to confirm its fetal nature (case 4, 116 fl; case 7, 140 fl; case 10, 135 fl). No local anesthetic was given, since in most cases only one puncture was needed. Bleeding from the punctured cord was closely observed. Fetal heart beat was monitored immediately after the procedure and the next day. Hemoglobin was electrophoresed by cellulose acetate membrane electrophoresis. The results were usually available the next day.

In cases where the cord insertion into the placenta was difficult to visualize, a segment of cord in a superficial amniotic fluid pouch or the intra-abdominal portion of the dilated umbilical vein was used for sampling (Fig. 1).

Results

Fetal blood samples were successfully obtained in all but one case (case 3). The details of fetal blood sampling and the results of hemoglobin electrophoresis are shown in Table II. Eight fetuses had a substantially high percentage of Bart's hemoglobin, warranting a diagnosis of Bart's hemoglobin hydrops fetalis. In these cases terminations were performed without delay to avoid maternal complications.

Case 2 had only 10% of Bart's hemoglobin in the aspirated cord blood; this was confirmed postnatally. This fetus was postulated to have homozygous α_2-thalassemia. Theoretically this would not result in hydrops fetalis. However, the couple already had a hydropic baby. They had normal mean corpuscular volumes, and careful antibody screening revealed no abnormalities. In spite of full-blown hydrops fetalis the thickness of the placenta (5 cm) and the amount of amniotic fluid remained normal in this case. These findings were different from those in Bart's hemoglobin hydrops fetalis. Since the cord blood contained only 10% Bart's hemoglobin, congestive heart failure of another cause was suspected. Maternal digitalization and furosemide therapy were tried, but the hydrops developed rapidly. Labor was induced 1 week later in the hope of salvaging the baby through aggressive postnatal management. However, the neonate died shortly after birth in spite of vigorous supportive measures. Postnatal fetal blood analysis revealed normal karyotype and no signs of intrauterine infection. Autopsy revealed no suggestive clues.

For case 3, although hemoglobin electrophoresis of the aspirated sample revealed pure maternal blood, no further attempts were made to obtain fetal blood. The patient was noted to have acute hepatitis with deepening jaundice. Spontaneous labor resulted in delivery of a hydropic baby who died minutes after birth. Cord blood analysis revealed 80% Bart's hemoglobin. The mother gradually recovered after delivery.

Case 1 was the only case without frank hydrops fetalis at the time of ultrasound-guided fetal blood sampling.

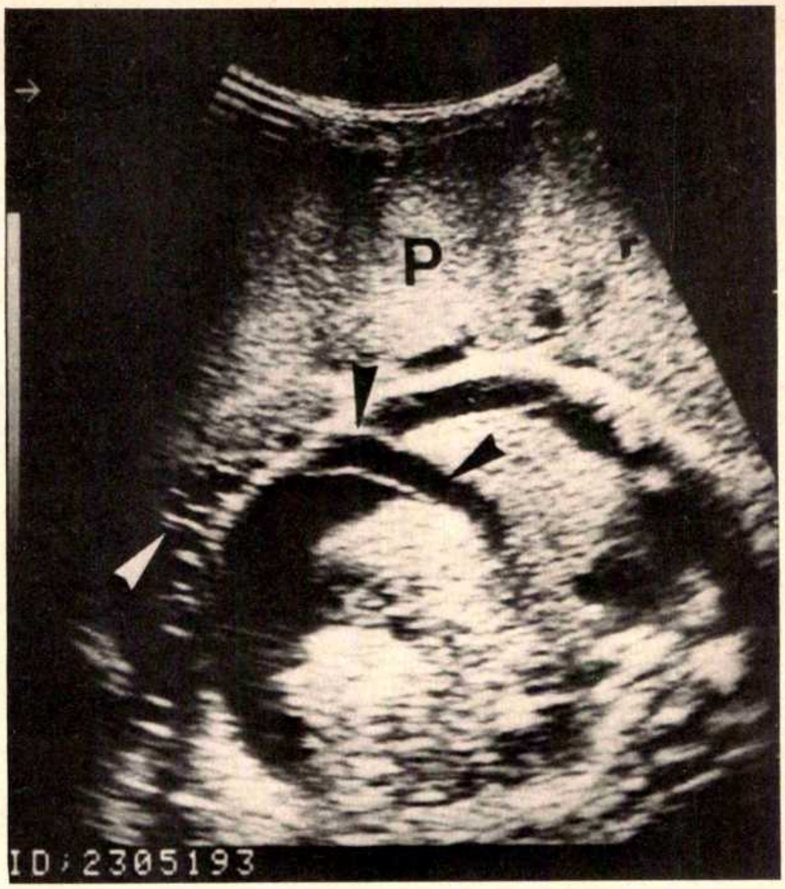

Fig. 1. In nonimmune hydrops fetalis with oligohydramnios an intra-abdominal portion of umbilical vein *(black arrow)* or a free cord loop *(white arrow)* can be used for needle insertion in ultrasound-guided fetal blood sampling. *P* denotes placenta.

The couple already had a normal child and a hydropic birth. No investigations on the previous hydropic birth were done. The mean corpuscular volumes of both parents were abnormally low. Ultrasound scanning at 21 weeks revealed a fetus with adequate growth. Polyhydramnios was the only abnormal finding (Fig. 2). In view of the history and the low mean corpuscular volumes of both parents, ultrasound-guided fetal blood sampling was performed, and the fetus indeed had Bart's hemoglobin hydrops fetalis. The pregnancy was terminated 3 weeks later. The delay was due to the parents' reluctance to accept the diagnosis. By that time fetal ascites was clearly visible. The fetal diagnosis was confirmed postnatally.

Significant obstetric complications were noted in 20% (two in 10) of this series. The most significant obstetric complication was noted in case 6. Emergency cesarean section was performed because of vaginal bleeding during induction of labor. Postoperative uterine bleeding necessitated a 2000 ml blood transfusion. Mild disseminated intravascular coagulation and aspiration pneumonia were also noted; these resolved after treatment. The patient recovered completely. The mother in case 10 had severe preeclampsia that resolved after delivery.

Bleeding from the punctured cord was observed in 20% of cases. Intrauterine fetal death occurred in two cases 1 and 3 days after ultrasound-guided fetal blood sampling. However, complete loss of baseline variability and severe decelerations of the fetal heart rate tracing were already present before ultrasound-guided fetal blood sampling.

Comment

With widespread use of anti-Rh immunoglobin, Rh isoimmunization has decreased markedly, and the diagnosis and management of nonimmune hydrops fetalis have become increasingly important. α-Thalassemia is one of the known causes of nonimmune hydrops fetalis and accounts for a substantial proportion of cases in Southeast Asia.[8-10] However, with increasing immigration this disease entity has become a worldwide health

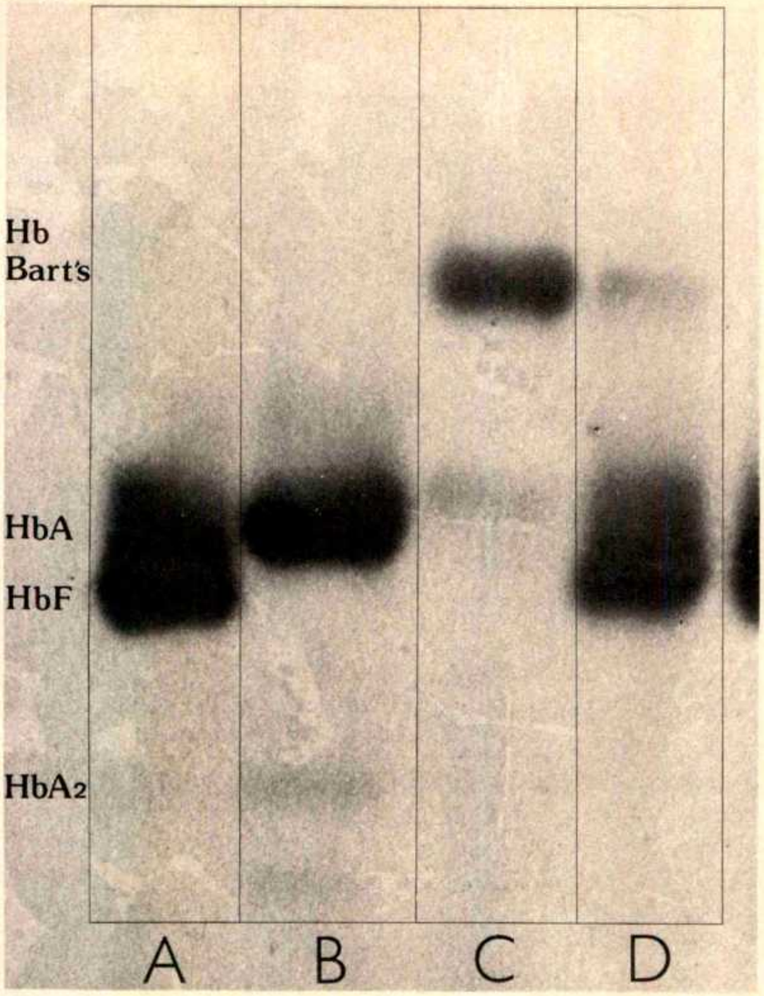

Fig. 2. Hemoglobin electrophoresis of fetal blood obtained from ultrasound-guided fetal blood sampling: *A*, normal fetal blood; *B*, maternal blood (case 3); *C*, Bart's hemoglobin hydrops fetalis; *D*, homozygous α_2-thalassemia (case 2).

problem. Guy et al.[6] experienced five cases in New York City during a 14-month period and called it an "emerging public health problem in the United States." Nakayama et al.[7] also described 18 cases in a Hawaiian hospital.

Ideally a screening program for high-risk women of Southeast Asian origin, such as that of Stein et al.,[11] could be applied to detect the possibly affected pregnancy at the first trimester. Chorionic villus sampling and DNA analysis can then be used for prenatal diagnosis. However, this is far from realization even in these Southeast Asian countries. Without screening, many affected cases of α-thalassemia will present as full-blown hydrops fetalis. The management clearly needs differentiation from that in nonimmune hydrops fetalis of other causes. Ultrasound-guided fetal blood sampling could provide a rapid answer in this situation. Some will of course present for prenatal diagnosis because of a history of hydrops fetalis. Ultrasound-guided fetal blood sampling will also be of help in some of cases, as it was in case 1 of our series. Although the fetus showed no sign of edema at 21 weeks, polyhydramnios alerted us to perform ultrasound-guided fetal blood sampling and confirmed the diagnosis well before hydrops fetalis developed.

In the α-thalassemia series of Liang et al.[10] 28% already had hydropic babies, whereas 40% in our series had hydropic infants. For those who have a history of hydrops, Liang et al.[10] proposed diagnosis in the early second trimester and termination of the affected pregnancy to avoid maternal morbidity. For cases of hydrops detected in the late second or early third trimester, both Guy et al.[6] and Liang et al.[10] recommended amniocentesis to obtain amniotic cells for α-globulin gene analysis. In our opinion chorionic villus sampling and DNA analysis should be recommended for those patients

Table II. Details of percutaneous fetal blood sampling, the outcome and complications in this series

Case	*Gestational age at UFBS (wk)*	*Cord insertion*	*Site of puncture*	*Through placenta*	*Cord bleeding*	*Blood aspirated and Hb analysis*
1	21	Clear	Cord insertion	No	No	3 ml fetal Bart's Hb 78.2%
2	31	Clear	Cord insertion	No	Yes	3 ml fetal Bart's Hb 10%
3	35	Clear	Free cord loop	No	No	Maternal blood
4	35	Obscured	Free cord loop	Yes	No	5 ml fetal Bart's Hb 96.9%
5	30	Obscured	Free cord loop	Yes	No	5 ml fetal Bart's Hb 80.3%
6	32	Obscured	Free cord loop	No	No	10 ml fetal Bart's Hb 93.7%
7	25	Obscured	Free cord loop	No	No	5 ml fetal Bart's Hb 96.5%
8	25	Obscured	Intra-abdominal portion, umbilical vein	Yes	No	4 ml fetal Bart's Hb 91%
9	31	Obscured	Intra-abdominal portion, umbilical vein	No	No	3 ml fetal Bart's Hb 85%
10	32	Obscured	Free cord loop	No	Yes	3 ml fetal Bart's Hb 99%

UFBS = Ultrasound-guided fetal blood sampling; Hb = hemoglobin; NND = neonatal death; IUFD = intrauterine fetal death; C/S = cesarean section; DIC = disseminated intravascular coagulation.

with a history of hydrops fetalis to exploit the advantage of a first-trimester diagnosis. For those cases detected at late second and early third trimester, ultrasound-guided fetal blood sampling and hemoglobin electrophoresis might offer a fast, definitive, and simple way of diagnosis, if the safety of ultrasound-guided fetal blood sampling can be further documented. Also, DNA restriction enzyme analysis of the amniotic cells involves the use of expensive enzyme and cumbersome radioisotope, which are not needed in the simple hemoglobin electrophoresis. In addition, the fetal blood can be used for other examinations such as rapid karyotyping and infection studies if hemoglobin electrophoresis excludes a α-thalassemia as the cause.

Nonimmune hydrops fetalis is frequently associated with a high rate of obstetric complications. In the series of Hutchinson et al.[1] 64% had difficulty in placental delivery or postpartum hemorrhage. Liang et al.[10] also reported that 50% involved manual placental removal and 33% required blood transfusion. In our series the much lower obstetric complication rate of 20% may be attributed to the rapid diagnosis by ultrasound-guided fetal blood sampling and termination without delay.

Nicolaides et al.[12] used fetoscopy to obtain a pure fetal blood sample in 30 cases of unexplained fetal hydrops. Ten had chromosomal abnormalities, one erythroleukemia, one α-thalassemia, and one cytomegalovirus infection. Our report, as did theirs, demonstrated the usefulness of a fetal blood sample in the assessment of fetal hydrops. Ultrasound-guided fetal blood sampling is easier than fetoscopy in obtaining fetal blood and perhaps carries a lower abortion risk as demonstrated by Daffos et al.[13] and Hobbins et al.[14] Furthermore, the fetal blood samples obtained are much better in terms of purity and quantity. We have encountered considerable difficulties in our first few cases in using a linear transducer with a central puncture channel. Shadowing of the missing crystal and poor contact were the major drawbacks. After switching to a sector scanner with a needle guide, sampling became much easier. The needle guide is a tremendous help in keeping the needle point and the target cord segment in line. Thus only depth control is necessary. However, even with a needle guide great effort is needed to keep the needle point and the target at the same plane. During puncture the patient should be asked to breathe lightly to avoid changing the puncture plane.

For ultrasound-guided fetal blood sampling in nonimmune hydrops fetalis, especially in α-thalassemia hydrops fetalis, either the crowded cord loops in an amniotic fluid pouch adjacent to the maternal abdominal wall or the intra-abdominal portion of the dilated umbilical vein may be used as puncture sites. The numerous paralleling cord segments sometimes made it difficult to differentiate the real umbilical vein and the intervening amniotic fluid space, since both are flanked by two umbilical arteries. A pulsed Doppler can be helpful in this situation, since flow signal will be detected only in real umbilical vein, not in the intervening amniotic fluid. Alternatively, trial and error can be used. If amniotic fluid is aspirated move the needle tip to the next space. Also, cord segment farther away from the chorionic plate should be used, since a dilated subchorionic blood space may be erroneously taken as a seg-

Outcome	*Obstetric complications*
Hysterotomy (placenta previa), NND	
Induction, NND	
Spontaneous labor, NND	Acute hepatitis
Induction, IUFD	
Induction, NND	
C/S (bleeding during induction)	Postpartum hemorrhage, DIC
Induction, IUFD	
Hysterotomy (previous C/S), NND	
Induction, NND	
Spontaneous labor, NND	Preeclampsia

ment of umbilical vein, and maternal blood may be obtained. This is the cause of our failure in case 3. Of course an immediate check of the mean corpuscular volume will avoid this mistake.

The two intrauterine fetal deaths that followed ultrasound-guided fetal blood sampling were thought not to be related to the procedure, since intrauterine fetal death is not uncommon in Bart's hemoglobin hydrops fetalis. Moreover, the fetus was in very poor condition as reflected by the fetal heart rate tracing.

Only two cases of bleeding (20%) from the punctured cord were noted in this series. This may have been due to use of the 22-gauge needle and the avoidance of traversing neighboring umbilical arteries. In our experience the blood jet is most likely to occur when a umbilical artery is traversed. Daffos et al.[13] used a 20-gauge needle and reported a 41% incidence of bleeding. The thinnest possible needle should be used to reduce bleeding, to avoid umbilical vein thrombosis, and to minimize maternal discomfort. We have recently tried a 25-gauge needle, as suggested by Hobbins et al.[14]

With a good experience in amniocentesis and a good sector scanner equipped with a needle guide, ultrasound-guided fetal blood sampling can be quickly mastered. As stated by Daffos et al.[13] this new procedure will open up an important field of investigation, and we believe it will play a key role in the management of the perplexing nonimmune hydrops fetalis. The safety of this relatively new procedure should of course be further verified.

REFERENCES

1. Hutchison AA, Drew JH, Yu VYH, et al. Non-immune hydrops fetalis: a review of 61 cases. Obstet Gynecol 1981;59:347.
2. Levine BJ, Gabbe SG. Nonimmune hydrops fetalis—how to improve survival. Contemporary Obstet Gynecol 1985; 25:43.
3. Landrum BG, Johnson DE, Ferrara B, et al. Hydrops fetalis and chromosomal trisomies. Am J Obstet Gynecol 1986;154:1114.
4. Mahony BS, Filly RA, Callen PW, et al. Severe nonimmune hydrops fetalis: sonographic evaluation. Radiology 1984;151:757.
5. Chen HY, Chow SN, Hsieh FJ, et al. Antenatal detection of hydrops fetalis by sonography: hemoglobin Bart's as a major etiological factor in Taiwan. J Ecog Med Ultrason 1984;5:321.
6. Guy G, Coady DJ, Jansen V, et al. Alpha-thalassemia hydrops fetalis: clinical and ultrasonographic considerations. Am J Obstet Gynecol 1985;153:500.
7. Nakayama R, Yamada D, Steinmiller V, et al. Hydrops fetalis secondary to Bart hemoglobinopathy. Obstet Gynecol 1986;67:176.
8. Lie-Injo LE. Hydrops fetalis with a fast-moving hemoglobin. Br Med J 1960;2:1649.
9. Wei PY, Chiang WT, Lin CC, et al. Prospective screening study of hemoglobin Bart's among newborn infants in Taiwan. J Assoc Obstet Gynecol Republic of China 1968;7:157.
10. Liang ST, Wong VCW, So WWK, et al. Homozygous alpha-thalassemia: clinical presentation, diagnosis and management. A review of 46 cases. Br J Obstet Gynaecol 1985;92:680.
11. Stein J, Berg C, Jones JA, et al. A screening protocol for a prenatal population at risk for inherited hemoglobin disorders: results of its application to a group of Southeast Asians and blacks. Am J Obstet Gynecol 1984;150:333.
12. Nicolaides KH, Rodeck CH, Lange I, et al. Fetoscopy in the assessment of unexplained fetal hydrops. Br J Obstet Gynaecol 1985;92:671.
13. Daffos F, Capella-Pavlovsky M, Forestier F. Fetal blood sampling during pregnancy with use of a needle guided by ultrasound: a study of 606 consecutive cases. Am J Obstet Gynecol 1985;153:655.
14. Hobbins JC, Grannum PA, Romero R, et al. Percutaneous umbilical blood sampling. Am J Obstet Gynecol 1985; 152:1.

Measurement of human fetoplacental blood volume in erythroblastosis fetalis

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The fetoplacental blood volume of the human fetus was measured with the change in hematocrit at the time of intravascular transfusion for severe erythroblastosis. A total of 121 measurements were made between 18 and 31 weeks' gestation. The volume ranged from 117 ml/kg at 18 weeks to 93.1 ml/kg at 31 weeks. These values compare closely with those reported for the sheep fetus and, when they are extrapolated to term, are similar to the blood volume of human newborns plus the residual placental blood volume. (AM J OBSTET GYNECOL 1987;157:50-3.)

Key words: Blood volume, fetal circulation, human fetus

Estimation of the fetoplacental blood volume of the human fetus and the change of this volume with gestational age would be of great interest as a basic physiologic parameter and has potential practical importance in the management of a number of fetal conditions. In suspected fetal bleeding, either external or fetal-maternal, one could better assess the significance of blood loss if the magnitude of hemorrhage could be related to fetal blood volume. Likewise, with diagnostic fetal blood sampling, the volume removed is more significant when expressed as a fraction of the circulating volume than as an absolute sample volume. In treating erythroblastosis fetalis with fetal transfusion, an estimate of normal circulating volume would be useful in both selecting the volume of donor blood and predicting the posttransfusion hematocrit value.

Although there are several reports of blood volume measurements in experimental animals, human data are limited to one study in which radioiodinated albumin was used in 19 human fetuses at the time of midtrimester hysterotomy pregnancy terminations.[1] The blood volume of newborn infants has been measured in a number of studies. However, the circulatory changes that accompany delivery and adaptation to neonatal life limit the degree to which these volumes can be extrapolated and applied to the fetus.

The treatment of severe erythroblastosis fetalis with intrauterine intravascular transfusion[2, 3] provides an opportunity to measure the circulating blood volume of the fetus using the change in fetal hematocrit values in response to transfusion with a specific volume of known hematocrit donor blood. This approach has been validated in animal studies in which there was close agreement between the values obtained with the hematocrit change with transfusion and the value obtained with ^{51}Cr-labeled red blood cells.[4] Furthermore, estimates of the blood volume in neonates with the change in hematocrit after partial exchange transfusion are in satisfactory agreement with published values obtained by indicator dilution procedures.[5]

In this article we will present the estimates of fetoplacental blood volume determined at the time of intrauterine intravascular transfusion based on 121 procedures between 18 and 31 weeks' gestation. Because all of the data needed for this determination are obtained routinely at the time of fetal transfusion, there was no additional risk to the fetus or mother.

Methods

Fetal transfusion. Fetoscopically directed intrauterine intravascular transfusions were carried out for severe erythroblastosis as described previously.[2, 3] Gestational age was determined by calculation from menstrual dates and confirmed by ultrasonographic measurement of the fetal biparietal diameter.[6] At the time of fetoscopy a fetal umbilical artery or vein was punctured and a sample of pure fetal blood (1 to 4 ml) obtained for hematologic and biochemical studies. Transfusion was then performed with packed red blood cells of known hematocrit value that had been cross-matched against the mother. The fetus was transfused with from 5 to 50 ml donor blood, depending on the gestational age and donor and fetal hematocrit values. At the end of the transfusion the needle was flushed with saline solution (0.5 ml), and after 60 seconds a sample of pure blood was aspirated. The first 0.5 ml aliquot of this was discarded. The fetal pretransfusion and posttransfusion hematocrit values and the hematocrit value of the donor blood were determined

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with a Coulter "S Plus" electronic cell counter (Coulter Electronics Inc., Hialeah, Florida). In some cases the posttransfusion hematocrit value was found to be <40%. In these cases additional blood was often infused, but data from this additional transfusion were not used in blood volume estimation. If there was any evidence of contamination of the fetal blood samples with amniotic fluid or difficulty obtaining the posttransfusion sample, the data were not used in the computation of fetoplacental blood volume. Data were available for analysis from 121 transfusions in 57 fetuses between 18 and 31 weeks' gestation.

Computation of blood volume. Blood volume estimation was based on the following assumptions: (1) Infused blood was thoroughly mixed with the circulating blood volume before obtaining the final fetal hematocrit sample. (2) The fetal blood volume at the end of the transfusion (V_f) was the sum of the initial fetoplacental blood volume (V_i) and the volume of the transfusion (V_d) less the volume of the initial fetal blood sample (V_s): $V_f = V_i + V_d - V_s$ (equation 1). (3) The red blood cell (RBC) volume in the fetal circulation at the end of the transfusion is equal to the sum of the initial hematocrit value (H_i) multiplied by the initial blood volume (V_i) and the volume of the transfusion (V_d) multiplied by the hematocrit value of the donor blood (H_d) less the volume of the sample (V_s) multiplied by the initial hematocrit value (H_i): RBC volume = $V_iH_i + V_dH_d - V_sH_i$ (equation 2). (4) The hematocrit value at the end of the transfusion (H_f) equals the final red cell volume divided by the final blood volume:

$$H_f = \frac{V_iH_i + V_dH_d - V_sH_i}{V_i + V_d - V_s} \qquad \text{Equation 3}$$

Equation 3 can be rearranged to give:

$$V_i = \frac{V_d(H_d - H_f) + V_s(H_f - H_i)}{H_f - H_i} \qquad \text{Equation 4}$$

All of the quantities on the right of equation 4 are measured at the time of fetal transfusion, so V_i, the initial fetoplacental blood volume, can be calculated.

Statistical analysis. Gestational age was rounded off to the nearest completed week of amenorrhea. Polynomial regression by the least-squares method was performed on an IBM Pc personal computer using a program written by Giuricin Matteo and Riccardo Brachelente of Florence, Italy.

Results

Fetoplacental blood volume. Fig. 1 shows the relationship of fetoplacental blood volume to gestational age in fetuses with erythroblastosis fetalis. Twenty-seven fetuses showed ultrasonographic evidence of hydrops fetalis at the time of their transfusion. In Fig. 1 the hydropic and nonhydropic fetuses are plotted together. As can be seen, there is no difference between the blood volume estimates for these two groups. The relationship of fetoplacental blood volume to gestational age was described better by a quadratic than a linear function. At 18 weeks the best estimate of fetoplacental blood volume, from the regression line, was 25.87 ml and at 31 weeks it was 151.7 ml.

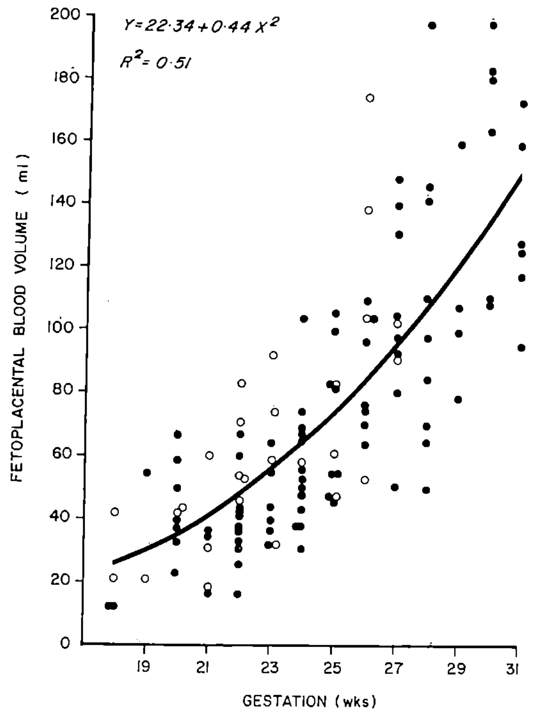

Fig. 1. Estimated fetoplacental blood volume. Open symbols represent hydropic fetuses.

In Fig. 2 the fetoplacental blood volume is expressed as milliliters per kilogram fetal weight. At 18 weeks' gestation the volume was 117 ml/kg and at 31 weeks it was 93.1 ml/kg. The overall mean was 101 ± 13.6 (2 × SD) ml/kg. The volume per kilogram fetal weight, computed in this way, was correlated with gestational age. Because fetal weight was not known for patients in this study at the time the blood volume was estimated, we used the fiftieth percentile for normal fetal weight as determined by Brenner et al.[7] and the blood volume estimate from the regression line in Fig. 1. Although these weights were derived for the patient population of Chapel Hill, North Carolina, and not the United Kingdom, it is unlikely that this would cause a significant error because the British population has a similar newborn weight distribution. The fetuses in this study had erythroblastosis fetalis, but ultrasonographic parameters of fetal growth, including head circumference, abdominal circumference (except in cases of hydrops fetalis), biparietal diameter, and femur length, were within the normal range for gestation.[8] Brenner et al. did find that there was significant variation in fetal

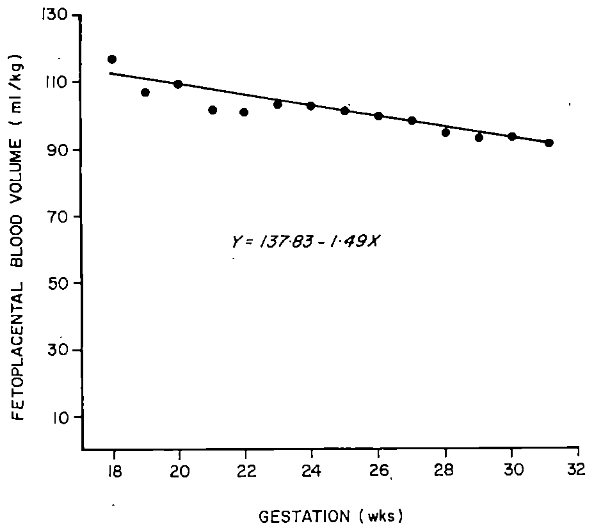

Fig. 2. Estimated fetoplacental blood volume expressed as milliliters per kilogram estimated fetal weight.

weight with fetal sex and maternal social class, but these effects were detected only after 36 weeks' gestation. Because our study involved fetuses <32 weeks' gestational age, it seems unlikely that there would be any significant difference between the weights of our fetuses and those reported by Brenner et al.

Comment

In this article we report the first estimates of human fetoplacental blood volume in continuing pregnancy. Using the change in fetal hematocrit values after intrauterine intravascular transfusion, we obtained values that are similar to those reported by Brace[4] for the sheep fetus and comparable with those for the human newborn and placenta after delivery.[9,10] Although newborn infants with erythroblastosis fetalis have expanded plasma and contracted red cell volumes, their total blood volumes are usually normal. In a few severe cases their blood volume is contracted.[11] Although we made no blood volume measurements in normal fetuses, we found that hydropic and nonhydropic fetuses had similar estimated blood volume.

The usual methods of determining blood volume that can be applied in adults or children cannot be used for the human fetus. Dye-dilution measurements require access to the circulation for the injection of the dye and then sampling of the blood at various times.[12] Furthermore, the safety and validity of such dye-dilution determinations have not been established for the human fetus. Techniques that entail use of radioactive labels, such as ^{51}Cr-labeled red blood cells, ^{131}I-albumin, or ^{131}I-fibrinogen, cannot be used because of the fetal radiation exposure. Stable isotope labeling of red cells with ^{50}Cr and subsequent activation to ^{51}Cr by neutron irradiation have been used in the newborn, but the necessary equipment is available in only a very few centers.[13]

In measuring volume by our technique we made a number of assumptions that may have introduced systematic errors. The first of these assumptions is that the mixing of donor blood with the fetoplacental blood is complete by 1 minute after the end of the transfusion. However, using ^{51}Cr-labeled red cells, Brace[4] has shown that after a single injection of labeled cells into a fetal vein, it took >5 minutes for complete mixing to occur. Our transfusions ranged in volume from 5 to 50 ml. They were infused at a rate of 1 to 3 ml/min and therefore the duration was approximately 10 to 50 minutes. Blood infused at the start of the transfusion had adequate time for mixing and only blood at the end of the transfusion would be subject to incomplete mixing. A sample taken at 1 minute might give an erroneously high hematocrit value and therefore a low blood volume estimate. Our second key assumption is that the blood volume at the end of the transfusion is equal to the initial volume plus the volume of donor blood less the volume of the fetal blood sample (equation 1). This is clearly an approximation because the fetal blood volume has been expanded substantially by the transfusion and will immediately begin to return toward normal. Brace[14] has studied the effect of rapid blood volume expansion in the sheep fetus using saline solution and iso-oncotic dextran 70. He found that with a dextran infusion amounting to approximately 15% of the fetal blood volume, there was 80% intravascular retention after 10 minutes and that this decreased to 55% by 30 minutes after the start of the infusion. This rate of plasma loss is more rapid than that reported for the adult.[15] Because we did not subject patients to delayed fetal blood sampling, we were unable to assess the rate of the reequilibration of the plasma volume in the human fetus. As noted above, fetal transfusions are carried out over time periods ranging up to 50 minutes. In general, larger transfusions took more time and were used in later-gestation fetuses. Because our computation assumes complete intravascular retention, any error as a result of plasma loss would result in an underestimate of the initial fetoplacental blood volume and this error would tend to increase with larger donor volumes and therefore advancing gestational age.

An error introduced by either or both of these assumptions could explain the discrepancy between our mean value, 101.3 ml/kg, and the value obtained by Morris et al.,[1] 162 ± 4.12 ml/kg. However, Brace[4] has pointed out that in the sheep fetus, radiolabeled proteins, albumin, and fibrinogen give larger apparent blood volumes than do labeled blood cells. Using ^{125}I-albumin and ^{125}I-fibrinogen, he obtained a value of 126 ml and 124 ml, respectively. He observed a very rapid loss of labeled protein from the fetal circulation immediately after injection. Since this resulted in a nonlinear semilogarithmic relationship of label concentration in the fetal blood to time, a true "time zero" value could not be obtained by extrapolation. Consequently, labeled proteins gave consistently larger blood volume estimates than did labeled red blood cells. He concluded that labeled proteins were inappropriate for estimating blood volume in the fetus. Morris et al. used ^{131}I-albumin and sampled fetal blood at two times and assumed a log-linear relationship with time in extrapolating to time zero. The blood volume Brace obtained with ^{51}Cr red cells was 110.3 ± 10.7 (SD) ml/kg fetal weight. Although the decline in blood volume per kilogram fetal weight we observed, from 117 ml/kg at 18 weeks to 93.5 ml/kg at 31 weeks, may be an artifact of the method of estimation, our mean value of 101.3 ± 6.8 (SD) ml/kg is not significantly different from Brace's value of 110.3 ± 10.7 (SD) ml/kg for the sheep fetus.

Reported blood volume for human newborns ranges from 66 to 110 ml/kg. This variability is dependent partly on the method of assessment and partly on the partition of blood between the infant and the placenta at delivery. Estimation of neonatal blood volume is fraught with most of the same problems encountered in estimation of fetal blood volume by indicator-dilution techniques. Delayed cord clamping and neonatal polycythemia have been associated with significantly higher neonatal blood volumes.[16, 17] Estimation of the residual placental blood volume has also been difficult. Some studies have simply measured the volume of blood that could be drained from the placenta after delivery.[16] Gruenwald[17] used the fetal hemoglobin concentration in placental homogenates and cord blood to estimate the placental blood volume. Both of these methods gave values ranging from 100 to 125 ml. If one assumes a term neonatal blood volume of 80 ml/kg and a term placental volume of 110 ml, a 3.3 kg fetus would be expected to have a fetoplacental blood volume of 374 ml or 113 ml/kg. This is remarkably similar to our value of 101 ml/kg and Brace's value[4] of 110 ml/kg.

In conclusion, in spite of a number of technical objections to our method of fetoplacental blood volume estimation, results have been obtained that are similar to those reported for chronic animal preparations and consistent with newborn plus placental blood volumes in humans assessed at delivery.

REFERENCES

1. Morris JA, Hustead RF, Robinson RG, Haswell GL. Measurement of fetoplacental blood volume in the human previable fetus. AM J OBSTET GYNECOL 1974;118:927.
2. Rodeck CH, Holman CA, Karnick K, Kemp JR, Whitmore DN, Austin MA. Direct intravascular fetal blood transfusion by fetoscopy in severe rhesus isoimmunisation. Lancet 1981;i:625.
3. Rodeck CH, Nicolaides KH, Warsoff SL, Fysh WJ, Gamsu HR, Kemp JR. The management of severe rhesus isoimmunisation by fetoscopic intravascular transfusion. AM J OBSTET GYNECOL 1984;150:769.
4. Brace RA. Blood volume and its measurement in the chronically catheterized sheep fetus. Am J Physiol 1983; 244:H487.
5. Rawlings JS, Pettett G, Wiswell TE, Clapper J. Estimated blood volumes in polycythemic newborns as a function of birth weight. J Pediatr 1982;101:594.
6. Campbell S. The assessment of fetal development by diagnostic ultrasound. Clin Perinatol 1974;1:507.
7. Brenner WE, Edelman DA, Hendricks CH. A standard of fetal growth for the United States of America. AM J OBSTET GYNECOL 1976;126:555.
8. Hohler CW. Ultrasound diagnosis of intrauterine growth retardation. In: Saunders RC, James AE, eds. Principles and practice of ultrasonography in obstetrics and gynecology. Norwalk, CT: Appleton-Century-Crofts, 1985: 157-73.
9. Usher R, Shephard M, Lind J. The blood volume of the newborn infant and placental transfusion. Acta Paediatr Scand 1963;52:497.
10. Yao AC, Lind J. Placental transfusion. Am J Dis Child 1974;127:128.
11. Brans YW, Milstead RR, Bailey PE, Cassady G. Blood-volume estimates in Coombs-test-positive infants. N Engl J Med 1974;290:1450.
12. Cassady G. Plasma volume studies in low birth weight infants. Pediatrics 1966;38:1020.
13. Faxelius G, Raye J, Gutberlet R, et al. Red cell volume measurements and acute blood loss in high-risk newborn infants. J Pediatr 1977;90:273.
14. Brace RA. Fetal blood volume response to intravenous saline solution and dextran. AM J OBSTET GYNECOL 1983;147:777.
15. Leonard JI, Abbreche PH. Dynamics of plasma-interstitial fluid distribution following intravenous infusion in dogs. Circ Res 1973;33:735.
16. Yao AC, Moinian M, Lind J. Distribution of blood between infant and placenta after birth. Lancet 1969;2:871.
17. Gruenwald P. The amount of fetal blood remaining in the placenta at birth. Proc Soc Exp Biol Med 1969; 130:326.

Estrogen, progesterone, prolactin, prostaglandin E_2, prostaglandin $F_{2\alpha}$, 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$, and 6-keto-prostaglandin $F_{1\alpha}$ gradients across the uterus in women in labor and not in labor

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Before or during labor in humans, changes in peripheral levels of estrogen and progesterone are not evident. Local alterations of estrogen, progesterone, and prolactin concentrations may be present and be accompanied by prostaglandin changes. The purpose of this study was to investigate the differences in concentrations of these hormones across the uterus and to evaluate their interrelationships in patients at term gestation with and without labor. Blood samples were obtained from a radial artery and a uterine vein in 22 women without and in 10 with labor. The difference between levels in the two blood vessels was designated as the gradient. Neither levels nor gradients were different between the two groups for estrone, estradiol, estriol, progesterone, or prolactin. The plasma levels of prostaglandin $F_{2\alpha}$, 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$, and prostaglandin E_2 were significantly increased in labor. Prostacyclin levels, as indicated by the 6-keto-prostaglandin $F_{1\alpha}$ metabolite, were not altered. The gradients for prostaglandin $F_{2\alpha}$ and E_2 were significantly increased in labor. The results of the study also suggested that, in gestation at term, serum prolactin is produced mainly by the pituitary and that estrone may originate from peripheral conversion of estradiol. We conclude that in humans prostaglandin gradients of the E and F groups are increased in labor. These increases are not associated with changes in sex steroids or prolactin. Prostacyclin metabolite gradients also appear not to be altered in labor, suggesting that some prostaglandins are selectively increased in early labor either by enhanced production or decreased metabolism or both. (AM J OBSTET GYNECOL 1987;157:54-8.)

Key words: Labor, uterine gradients, sex steroids, prostaglandins

In a variety of animals the mechanisms involved in the initiation of labor have been partially elucidated. In rodents, progesterone rises both in the blood and at the level of the uterus during pregnancy and then falls prior to labor, while estrogens continue to increase so that just before delivery the ratio of estrogen to progesterone is high. Loss of the protective effects of progesterone and the resulting estrogen dominance will then increase the production and release of prostaglandins and enhance the contractility of the myometrium. Administration of estrogens to these animals will induce uterine contractions and progesterone will block labor. In sheep, the changes in those steroids are preceded by a sudden rise in fetal adrenocorticoids and premature labor will start after administration of adrenal steroids to the fetus. Conversely, ablation or blockage of the adrenal gland of the fetal sheep will lead to prolonged pregnancy. In humans, adrenocorticoid stimulation does not seem to be involved in the initiation of labor. However, in anencephaly, where adrenocortical function is severely impaired, delivery is generally delayed. Prolonged postmaturity is also seen in patients with placental sulfatase deficiency.[1] Both conditions will cause a large decrease in the fetal contribution to circulating estrogens. In the human, levels of estrogen and progesterone in peripheral blood do not exhibit the changes found in the animals as described above. Thus the simple block by progesterone of the stimulation of the myometrium by estrogen does not seem to play a direct role in labor and delivery.[2]

Prolactin has been shown to have a stimulating effect on uterine contractility.[3] During pregnancy the circulating levels of prolactin rise gradually, although the concentrations are much higher in the amniotic fluid. This hormone is produced by the pituitary but also at

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the level of the uterus by the decidua. The synthesis and production of decidual prolactin, in contrast to the prolactin originating from the pituitary, are not regulated by dopamine. Around parturition there is a multiphasic pattern of the circulating blood levels, suggesting a role of prolactin in labor and delivery.[4]

Prostaglandins of the E and F groups are known to stimulate uterine contractions and their role in the initiation of parturition is likely to be an important one. This is suggested by the fact that prostaglandin inhibitors can delay the onset of labor and prevent or arrest premature labor. Levels in amniotic fluid and circulating blood of the metabolites of these prostaglandins are elevated in labor but the exact origin of these compounds in unclear. The role and origin of prostacyclin in human parturition also is not known; this hormone may produce both uterine and vascular muscle relaxation.[5]

The present study was undertaken to evaluate the source of prostaglandin E_2 and $F_{2\alpha}$ and prostacyclin in term pregnancy and assess the role of these substances in labor and the interrelationships with estrogens, progesterone, and prolactin. The contribution of the uterofetoplacental unit was evaluated by determining levels in the uterine vein and the radial artery. The difference in concentrations between these two sites was designated the gradient. On the basis of the evidence obtained in animals it was hypothesized that levels and gradients of all hormones would be elevated in the patients in labor, with the possible exception of those of progesterone.

Material and methods

Subjects. A total of 32 patients were included in the study, after informed consent was obtained. All of them were between 38 and 42 weeks of gestation by clinical estimation and, in most cases, early ultrasonographic examination. Twenty-two patients underwent a cesarean section without any signs of labor. Ten had cesarean section performed while in early active labor for abnormal presentations or were scheduled for an elective primary or repeat cesarean section and presented with regular contractions and increasing cervical dilatation and/or effacement. The presence or absence of labor was demonstrated with the use of electronic tocometric monitoring. None of the patients in either group had a cervical dilatation of >4 cm. No patient in labor was stimulated with oxytocin or demonstrated a dysfunctional labor pattern. All patients underwent operation while under epidural anesthesia and had no other medical or obstetric diseases or complications.

Specimen collection. After the peritoneal cavity was entered but before the uterus was incised, a simultaneous blood sample was obtained from a radial artery and a uterine vein. Blood samples were kept on ice and serum or plasma was separated without delay by centrifugation at 4° C and 1500 × g for 10 minutes. Samples were stored at −20° C until assay. Evaluations of hormone concentrations in both uterine veins in a limited number of patients showed similar values, irrespective of the location of the placenta, so that only one side was sampled for the study.

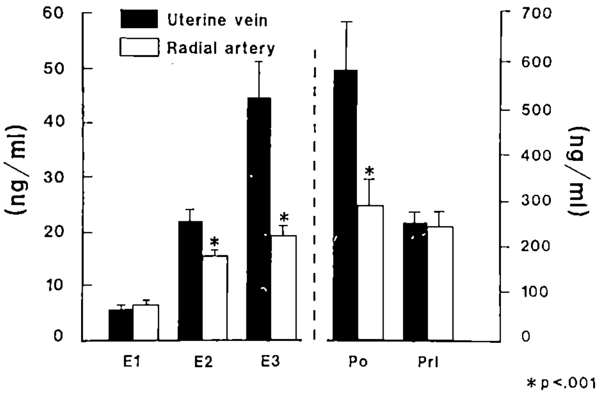

Fig. 1. Mean ± SE of levels of estrone *(E1)*, estradiol *(E2)*, estriol *(E3)*, progesterone *(Po)*, and prolactin *(Prl)* in the uterine vein *(solid bars)* and the radial artery *(open bars)* in all patients.

Hormone assays. For each hormone, samples were measured in the same assay. Estrone, estradiol, and progesterone determinations were performed by radioimmunoassay techniques after extraction with anhydrous ethyl ether and separation of fractions on a Celite column. Estriol was assayed with a Coat-A-Count Free Estriol kit (Diagnostic Products Corporation, Los Angeles, California). Serum prolactin was measured with Kit No. 125 purchased from Radioassay Systems Laboratories, Inc., Carson, California. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), 13, 14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ (PGFM), prostaglandin E_2 (PGE_2), and 6-keto prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), a stable metabolite of prostacyclin, were determined by sensitive and specific antisera and modified assay procedures, after acidified plasma was extracted with diethyl ether. For all hormone determinations the interassay coefficient of variation was <13%.

Statistical analysis. Results are presented as mean ± SEM. Data were evaluated by Student's *t* test. Unpaired *t* testing was used to compare levels and gradients of hormones between patients in labor and those without contractions. Paired *t* testing was used to assess differences between hormone levels in the uterine vein and radial artery. Correlations between hormone levels were determined by regression analysis.

Results

Levels of total unconjugated estrone, estradiol, estriol, progesterone, and prolactin in the uterine vein and the radial artery in all patients are shown in Fig. 1. The concentrations of estradiol, estriol, and pro-

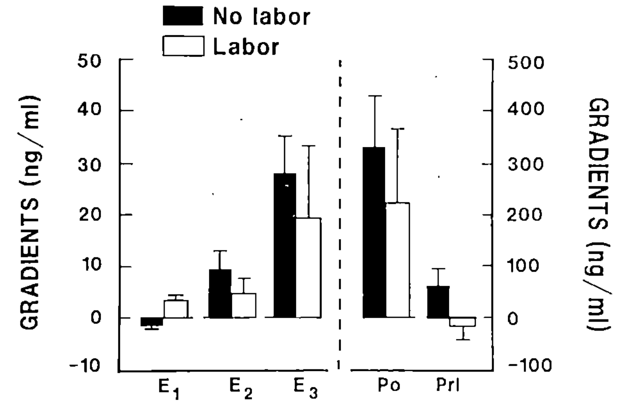

Fig. 2. Mean ± SE of gradients of estrone, estradiol, estriol, progesterone, and prolactin in patients with labor *(open bars)* and without labor *(closed bars)*.

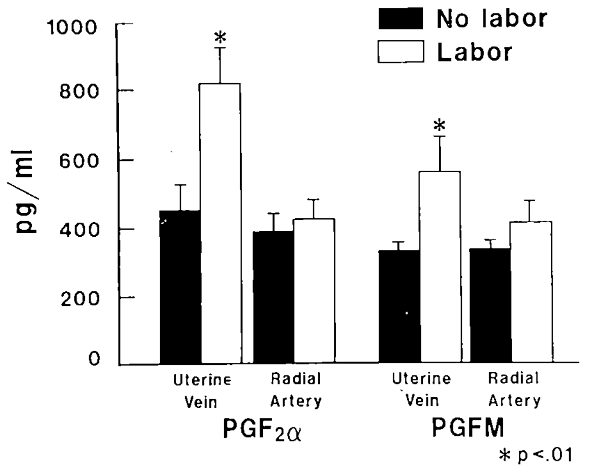

Fig. 3. Mean ± SE of levels of $PGF_{2\alpha}$ and PGFM in the uterine vein and radial artery in patients with labor *(open bars)* and without labor *(closed bars)*.

gesterone were significantly higher in the uterine vein ($p < 0.001$). However, serum estrone and prolactin levels in the uterine vein were not increased. There were no statistically significant correlations between levels of any of these hormones.

Fig. 2 depicts the gradients of estrone, estradiol, estriol, progesterone, and prolactin in patients with and without labor. Because of the variability of values, the standard errors are fairly large, and gradients appear to be somewhat greater in the patients without labor, however, without reaching statistical significance.

Fig. 3 represents the concentrations of $PGF_{2\alpha}$ and PGFM in the uterine vein and radial artery in patients with and without labor. In the uterine vein levels of these prostaglandins were significantly higher in the patients in labor ($p < 0.01$). No significantly different levels were found in the radial artery.

Fig. 4 shows the mean and standard error of the concentrations of PGE_2 and 6-keto-$PGF_{1\alpha}$ in the uterine vein and radial artery in patients with and without labor. In the uterine vein, levels of PGE_2 were significantly higher in the patients in labor ($p < 0.005$). No significantly different levels were found in the radial artery.

Fig. 5 depicts gradients of $PGF_{2\alpha}$, PGFM, PGE_2, and 6-keto-$PGF_{1\alpha}$ in patients with and without labor. In the patients in labor, there was a significantly increased gradient of $PGF_{2\alpha}$ and PGE_2 ($p < 0.03$ and 0.05, respectively.

There were no significant correlations between any of the sex steroids or prolactin and any of the prostaglandins, nor were there significant correlations between the ratios of the estrogens to progesterone and the prostaglandins.

Comment

This study confirms the importance of the fetus and placenta in the production of estradiol, estriol, and progesterone at term. Labor does not change the circulating levels of these hormones. Prostaglandins of the F and E groups are produced in higher concentrations in women in labor.

The differences in findings between experimental animals and humans as far as the changes in the peripheral circulation of hormones and their relation to initiation of labor make extrapolation from animal data very difficult.[1] The in vivo human model used in this study compares concentrations of hormones in the uterine vein with those in the radial artery. This gradient allows insight into the contribution of the uterus, the placenta, and the fetus to circulating levels. Depending on the origin of the different hormones, the gradient is a reflection of fetal and placental production for all hormones except 6-keto-prostaglandin $F_{1\alpha}$, the gradient of which reflects the production of the uterus, fetus, and placenta. The accuracy of the gradient in representing this production would be particularly true for compounds such as the sex steroids and prolactin since they have a relatively long half-life. For the prostaglandins with their rapid metabolism, especially in the lung, the gradient is at least an approximation of uterofetoplacental contribution.

The role of the ratio between estrogen and progesterone in the initiation of labor could be a very important one because of its effect on prostaglandin production. Progesterone withdrawal also would induce the formation of gap junctions in the myometrium, promoting synchronization and coordination of uterine contractility.[1] Most authors, but not all, examining peripheral levels of estrogen and progesterone and their ratio in women in labor and those without contractions did not find any differences, in contrast to what was found in many animal species.[5] In one report a significant decrease of concentrations of progesterone before

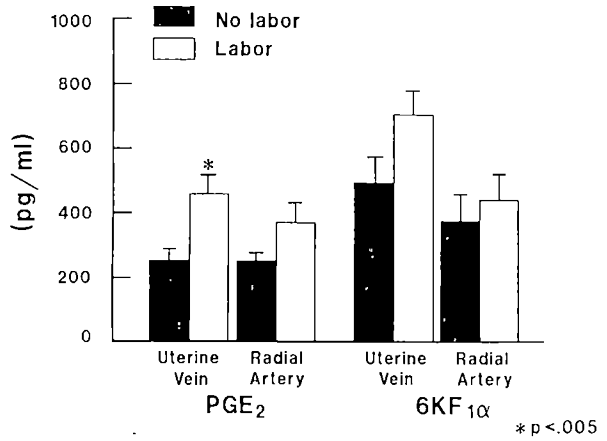

Fig. 4. Mean ± SE of levels of PGE_2 and 6-keto-$PGF_{1\alpha}$ in the uterine vein and radial artery in patients with labor *(open bars)* and without labor *(closed bars)*.

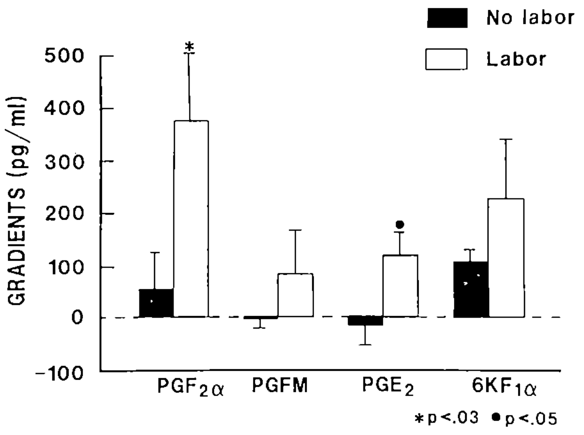

Fig. 5. Mean ± SE of gradients of $PGF_{2\alpha}$, PGFM, PGE_2, and 6-keto-$PGF_{1\alpha}$ in patients with labor *(open bars)* and without labor *(closed bars)*.

labor in the uterine vein but not in a peripheral vein was found.[6] This finding could not be confirmed in the present study, in which no differences of the fetoplacental contribution to circulating levels of estrogens, progesterone, and prolactin between patients in labor and those not in labor were seen. Ratios also were not different. This makes a mechanism such as described in rodents and sheep unlikely but local effects may be involved. The existence of local mechanisms was not addressed in our study.

We found that there was no contribution of estrone by the fetoplacental unit to circulating levels, suggesting that concentrations of this estrogen could be the result of peripheral conversion of estradiol. This may explain the parallelism in concentrations of estradiol and estrone during pregnancy as described by Lauritzen and Klopper.[7] Another explanation is the possibility that the fetoplacental unit extracts as much estrone from the circulation as it produces.

In pregnancy, there is maternal prolactin production from two sources: the pituitary[8] and the decidua.[9] It is uncertain which source contributes most to circulating levels. Ablation of the pituitary or suppression of pituitary lactotropes by bromocriptine administration in pregnancy causes a large decrease in maternal serum prolactin concentrations.[10] In vitro studies indicate that the decidual production of prolactin is most prominent in the second trimester.[11] In this study we found no significant prolactin contribution from the fetoplacental unit to circulating levels or changes in peripheral or uterine concentrations in patients with or without labor. This finding confirms that the majority of this hormone in pregnancy is produced by the pituitary. It also suggests that uterine prolactin produced by the decidua is secreted directly and preferentially into the amniotic fluid.

This study further substantiates that during labor prostaglandins in both the F group and the E group are increased in concentration. This increase is important, since Giannopoulis et al.[12] have shown that the concentration and affinity of prostaglandin E receptors do not change at parturition in humans. Both $PGF_{2\alpha}$ and PGE_2 are very potent stimulators of uterine contractions and are produced in the amnion and/or the decidua.[13] Their amniotic fluid concentrations are known to be elevated in women in labor. The use of the gradients adds convincing evidence that the major source of these prostaglandins is indeed the fetoplacental unit.

This increase of $PGF_{2\alpha}$ and PGE_2 could be the result of enhanced production or reduced catabolism of these compounds, or a combination of these mechanisms could play a role. Gradients of PGFM were not found to be elevated. This metabolite of $PGF_{2\alpha}$ is reported to be increased only in the presence of advanced cervical dilatation of ≥6 cm.[14] The patients in our study were all in early active labor and none had reached this degree of dilatation. The stimulus for the increased production of prostaglandins could not be determined by this study, but levels or ratios of estrone, estradiol, estriol, progesterone, or prolactin did not seem to be involved in a direct fashion, since there were no correlations between levels or ratios of these hormones and the prostaglandins.

The role in labor of prostacyclin, which is also produced in the myometrium, could be related to its ability to relax blood vessels and prevent intravascular coagulation.[15] Since both $PGF_{2\alpha}$ and PGE_2 cause profound vasoconstriction, prostacyclin could be involved in a mechanism securing adequate blood supply to the placenta during a uterine contraction.[5] However, there was no significant increased contribution by the uterofetoplacental unit during labor, although the differences between levels in the radial artery and the uterine vein in patients in labor indicated a trend toward a higher gradient. The work of Makarainen and Ylikorkala[16] in amniotic fluid shows that there is a continuing increase of 6-keto-PGF$_{1\alpha}$ concentrations as cervical dilatation progresses. Our results do suggest that prostacyclin is not involved in the initiation of parturition.

We conclude that at term gestation the fetoplacental unit produces large amounts of estradiol, estriol, and progesterone, but labor does not seem to change this production or alter the ratios of these sex steroids, as is seen in animals. There is no contribution to circulating estrone and prolactin levels by the fetoplacental unit. PGF$_{2\alpha}$ and PGE$_2$ gradients are enhanced in women in labor, suggesting increased production or decreased catabolism or both by the fetoplacental unit. Prostacyclin gradients are unchanged during labor, indicating that the role of this compound in the initiation of human parturition may be limited.

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REFERENCES

1. Ryan KJ. Maintenance of pregnancy and the initiation of labor. In: Tulchinsky D, Ryan KJ, eds. Maternal-fetal endocrinology. Philadelphia: WB Saunders, 1980:297.
2. Hartikainen-Sorvi A-L, Kauppila A, Tuimala R, Vinikka L, Ylikorkala O. The lack of significant change in plasma progesterone and estradiol-17β levels before the onset of human labor. Acta Obstet Gynecol Scand 1981; 60:497.
3. Bigazzi M, Nardi E. Prolactin and relaxin: antagonism on the spontaneous motility of the uterus. J Clin Endocrinol Metab 1981;53:665.
4. Rigg LA, Yen SSC. Multiphasic prolactin (PRL) secretion during parturition in humans. AM J OBSTET GYNECOL 1977;128:215.
5. Fuchs A-R, Fuchs F. Endocrinology of human parturition: a review. Br J Obstet Gynaecol 1984;91:948.
6. Drover JW, Casper RF. Initiation of parturition in humans. Can Med Assoc J 1983;128:387.
7. Lauritzen C, Klopper A. Estrogens and androgens. In: Fuchs F, Klopper A, eds. Endocrinology of pregnancy. Philadelphia: Harper & Row, 1983:73.
8. Josimovich JB, Weiss G, Hutchinson DL. Source and disposition of pituitary prolactin in maternal circulation, amniotic fluids, fetus and placenta in the pregnant rhesus monkey. Endocrinology 1974;94:1364.
9. Riddick DH, Kusmik WF. Decidua: a possible source of amniotic fluid prolactin. AM J OBSTET GYNECOL 1977; 127:187.
10. Bigazzi M, Ronga R, Lancranjan I, et al. A pregnancy in an acromegalic woman during bromocriptine treatment: effect on growth hormone and prolactin in the maternal, fetal and amniotic compartments. J Clin Endocrinol Metab 1979;48:9.
11. Liu TI, Minaguchi H, Taga M, Moti H, Sakamoto S. Synthesis of prolactin by human decidua in vitro. Endocrinology 1981;28:391.
12. Giannopoulis G, Jackson K, Kredentser J, Tulchinsky D. Prostaglandin E$_2$ and F$_{2\alpha}$ receptors in human myometrium during the menstrual cycle and in pregnancy and labor. AM J OBSTET GYNECOL 1985;153:904.
13. Okazaki T, Casey ML, Okita JR, MacDonald PC, Johnston JM. Initiation of human parturition. XII. Biosynthesis and metabolism of prostaglandins in human fetal membranes and uterine decidua. AM J OBSTET GYNECOL 1981;139:373.
14. Husslein P, Fuchs A-R, Fuchs F. Oxytocin and the initiation of labor. I. Prostaglandin release during induction of labor by oxytocin. AM J OBSTET GYNECOL 1981; 141:688.
15. Jeremy JY, Barradas MA, Craft IL, Michailidis DP, Dandona P. Does human placenta produce prostacyclin? Placenta 1985;6:45.
16. Makarainen L, Ylikorkala O. Amniotic fluid 6-keto-prostaglandin F$_{1\alpha}$ and thromboxane B$_2$ during labor. AM J OBSTET GYNECOL 1984;150:765.

Contraceptive use, pelvic inflammatory disease, and fertility problems among American women: 1982

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To examine the relationships between contraceptive use, pelvic inflammatory disease, and fertility problems, we analyzed data from the 1982 National Survey of Family Growth. This nationally representative sample of reproductive age women in the United States provides the first population-based estimates of fertility problems and pelvic inflammatory disease. Self-reported pelvic inflammatory disease was higher among whomen who had ever used an intrauterine device (23%) than among those who had ever used oral contraceptives (17%). Controlling for other variables, fertility problems were most common among women who had never used a method (42%), but they were also more common among oral contraceptive users (26%) than intrauterine devices users (17%). Our findings are consistent with the hypotheses that (1) intrauterine devices were used primarily by women with proved fertility and (2) oral contraceptives were associated with pelvic inflammatory disease in complex ways, although the exact nature of this association and the underlying disease are not yet completely understood. (AM J OBSTET GYNECOL 1987;157:59-64.)

Key words: Oral contraceptives, intrauterine device, pelvic inflammatory disease, infertility

Contraceptive choice has an important influence on the likelihood of pelvic inflammatory disease and resultant fertility problems. Users of intrauterine devices are at higher risk for the development of pelvic inflammatory disease than nonusers,[1] and they also have a greater likelihood of tubal infertility.[2, 3] Although oral contraceptive use has been found to protect against pelvic inflammatory disease,[4] this protective effect may be organism specific, largely influencing symptomatic gonococcal pelvic inflammatory disease.[5] In fact, accumulating evidence suggests that oral contraceptives may even promote chlamydial cervicitis,[6] and their effect on chlamydial pelvic inflammatory disease is unclear. A recent study suggests a slightly higher risk for primary tubal infertility among users of oral contraceptives compared with nonusers.[3] Nevertheless, empiric evidence on the relationship between oral contraceptive use and tubal infertility is scant and far from conclusive.

These epidemiologic findings have shed considerable light on pelvic inflammatory disease and infertility risks associated with contraceptive choice, but data on the public health impact of such risks have not been available. Although temporal trends in contraceptive use,[7] pelvic inflammatory disease,[8] and infertility[9] in the United States have been described, until the present study, no single source of data has contained all the critical variables to study the relationships among contraceptive use, pelvic inflammatory disease, and fertility problems. In this article, we present the first population-based national estimates of these relationships from a representative sample of American women.

Methods

We obtained data for this analysis from the National Survey of Family Growth, Cycle III, conducted in 1982 by the National Center for Health Statistics. The National Survey of Family Growth is a periodic survey of reproductive-age women designed to provide information on factors affecting childbearing and maternal and child health.[9] The 1982 survey was based on personal interviews with a multistage area probability sample of 7969 women between 15 and 44 years of age in the United States. We limited this analysis to women who had ever had intercourse and who were not sterilized for contraceptive reasons or married to contraceptively sterile men. The sample size for this group was 5504 women including 1308 women (24%) who had fertility problems.

The 1982 survey included two questions on pelvic inflammatory disease. The respondent was first asked whether she had ever been treated in a doctor's office,

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Table I. Definition and number of fertility problems in the United States from the 1982 National Survey of Family Growth Cycle III

Term	*Definition*	*Estimated numbers* (million)*
Fertility problems		9.2
Surgically sterile for health reasons	Sterility resulting from operation in which contraception was not a contributing reason	4.2
Infertile	12 mo or more of unprotected intercourse without conception	2.4
Impaired fecundity		4.5
Nonsurgically sterile	Self-reported inability to have a baby	0.9
Subfecund	Self-reported difficulty or danger in conceiving or carrying to term	3.0

*It is possible for women to be classified as both infertile and impaired fecundity. Therefore the sum of the components is larger than the total with fertility problems.

Table II. Self-reported pelvic inflammatory disease by parity and contraceptive choice: American women, ages 15 to 44 years, 1982

	Contraceptive choice	
Parity	*Oral contraceptive ever*	*Intrauterine device ever*
All	16.8	23.3‡
0	12.3	21.1†
1+	19.3*	23.8†§

*Difference by parity significant at $p < 0.01$.

†Difference by parity not significant.

‡Difference by method significant at 0.01.

§Difference by method significant at 0.10.

Note: As noted in the text, the results in Tables II to VII are percentages of U.S. women 15 to 44 years of age in 1982 (excluding those who have never had intercourse and those who are sterilized for contraceptive reasons). The percentages are weighted and are national estimates. The complementary category for each percentage (e.g., those not reporting pelvic inflammatory disease) may be obtained by subtracting the percentage from 100. Thus the percentage of oral contraceptive users who did not report pelvic inflammatory disease was 100% − 16.8%, or 83.2%. These complementary categories are not shown to save space and to make the tables easier to read.

clinic, or emergency room for an infection in her fallopian tubes, womb, or ovaries. The diagnosis was further clarified by use of the phrase ". . . also called a pelvic infection, pelvic inflammatory disease, or PID." The respondent was next asked how many different times, if ever, she had been hospitalized for a pelvic infection. The data on pelvic inflammatory disease have been reported elsewhere.[10]

For the present analysis, we defined couples with fertility problems to include those infertile, surgically sterile for health reasons, and those with impaired fecundity. Couples were classified as infertile if neither spouse was surgically sterilized, the couple did not use any form of contraception for 12 months or more of continuous marriage, and they did not conceive during the 12-month period. Couples were classified as subfecund in case of self-reported difficulty or danger to conceive and/or carry a pregnancy to term. The term "impaired fecundity" was used to describe the sum of the nonsurgically sterile and subfecund categories. The derivation of these categories has been explained in more detail elsewhere.[9]

We used t tests to test the significance of relationships among the variables studied here. To examine the relationships of pelvic inflammatory disease, parity, and contraceptive choice simultaneously while controlling for age, we used multiple classification analysis. The standard errors and t tests used in this article used the formulas and procedures recommended for this survey.[11] These formulas are based on the balanced half-sample replication technique, an accurate method for estimating sampling errors from complex samples such as this one. These estimates take into account both the sample size and sample design on which our weighted percentages are based. The significance tests in our multivariate analyses are also based on the recommended formulas and do not assume simple random sampling.

National estimates

Our data in this study were weighted to represent the 37.2 million women in the United States in 1982 who were between 15 and 44 years of age, had had intercourse, and were not contraceptively sterile, including 9.2 million women with fertility problems.[9] The weight for each case was adjusted for the different sampling rates among whites, blacks, and teenagers and was further adjusted for nonresponse to produce national estimates.

In 1982, 9.2 million women had fertility problems, including 2.3 million who were childless and 6.9 million with one or more children. Of these 9.2 million, 4.2 million were surgically sterile for noncontraceptive health reasons, and 5.0 million had other fertility problems, such as self-reported difficulty in conceiving or

Table III. Fertility problems by parity and self-reported pelvic inflammatory disease: American women, ages 15 to 44 years, 1982

	Self-reported pelvic inflammatory disease		
Parity	*Ever treated*	*Never treated*	*Total*
All	44.2*	21.2	24.7
0	32.2*	13.3	15.2
1+	48.8*	27.2	31.2

*Difference by whether ever treated for pelvic inflammatory disease significant at $p < 0.01$.

carrying to term or a year or more of unprotected intercourse without a pregnancy (Table I). About 7.6 million women had had pelvic inflammatory disease at some time in their lives.[10]

Results

The percentage of women reporting that they had had pelvic inflammatory disease was higher among those who had at some time in their lives used an intrauterine device (23%) compared with those women who had used oral contraceptives (17%). This difference was twice as large for childless women (21% versus 12%) as for women with children (23% versus 19%) (Table II). Women who had one or more births also had higher pelvic inflammatory disease rates than did childless women, and this difference was larger among oral contraceptive users ($p < 0.01$) than among intrauterine device users where it was not significant.

The association between pelvic inflammatory disease and fertility problems was striking. Among women who were treated for pelvic inflammatory disease, the proportion with fertility problems (44%) was more than twice as high as among women who were not treated for pelvic inflammatory disease (21%) ($p < 0.01$) (Table III). This difference was observed among both childless women (32% versus 13%, $p < 0.01$) and women with children (49% versus 27%, $p < 0.01$).

Moreover, the effect of pelvic inflammatory disease on fertility problems was found among both oral contraceptive and intrauterine device users (Table IV). Those women who had been treated for pelvic inflammatory disease were twice as likely ($p < 0.01$) to have fertility problems as those who had not been treated for pelvic inflammatory disease (38% versus 21% among intrauterine device users and 42% versus 21% among oral contraceptive users) (Table IV). Among childless oral contraceptive users, 29% of those women treated for pelvic inflammatory disease had fertility problems compared with just 13% of those women never treated for pelvic inflammatory disease. The difference among childless intrauterine device users was not significant, primarily because it was based on a smaller number of cases. The differences in the percentage with fertility problems between intrauterine device and oral contraceptive users were small among those who had never been treated for pelvic inflammatory disease. However, among those who had been treated for pelvic inflammatory disease, oral contraceptive users were more likely to have fertility problems than were intrauterine device users (42% and 38%, respectively). Although not statistically significant, this difference was observed overall and was consistent in both parity categories (Table IV).

To determine whether hospitalized (as opposed to ambulatory) treatment of pelvic inflammatory disease was associated with greater fertility problems, we compared rates of fertility problems among women who had been hospitalized for this condition, to those who had received only ambulatory care (Table V). Women who had been hospitalized for pelvic inflammatory disease treatment were far more likely to have had fertility problems (57%) than were women treated in an ambulatory setting (39%). Although the difference between hospital and ambulatory treatment categories was rather small among childless women, it was very large (64% compared with 42%) and statistically significant ($p < 0.01$) among women with one or more births.

Women who had been hospitalized for pelvic inflammatory disease were more likely to have fertility problems than were those treated in ambulatory settings.

Table IV. Fertility problems by parity, contraceptive choice, and self-reported pelvic inflammatory disease: American women, ages 15 to 44 years, 1982

	Self-reported pelvic inflammatory disease		
Contraceptive choice	*Ever treated*	*Never treated*	*Total*
Intrauterine device ever			
Parity			
All	37.8*	21.3	25.2
0	26.6†	15.3	17.7
1+	39.8*	22.6	26.7
Oral contraceptive ever			
Parity			
All	42.0*	20.5	24.1
0	29.4‡	12.9	14.9
1+	46.6†	25.3	29.4

*Difference by pelvic inflammatory disease significant at $p < 0.01$.

†Difference by pelvic inflammatory disease not significant.

‡Difference by pelvic inflammatory disease significant at $p < 0.05$.

Table V. Fertility problems by parity and by hospitalized and ambulatory pelvic inflammatory disease: American women ages 15 to 44 years, 1982

	Self-reported pelvic inflammatory disease		
Parity	*Ambulatory*	*Hospitalized*	*Any pelvic inflammatory disease*
All	38.5*	57.3	44.2
0	31.3	35.1	32.3
1+	41.6*	63.9	48.8

*$p < 0.01$.

This was true among both users of intrauterine devices and oral contraceptives (Table VI). Women who had used oral contraceptives and had been hospitalized for pelvic inflammatory disease were far more likely to have fertility problems than were oral contraceptive users who received ambulatory treatment for pelvic inflammatory disease (55% versus 37%). This difference was statistically significant ($p < 0.01$). Women who had used intrauterine devices and had been hospitalized for pelvic inflammatory disease were also more likely to have fertility problems (45% versus 35%) than intrauterine device users who were treated in ambulatory settings. This difference was not statistically significant because the number of women who had used intrauterine devices was much smaller than those who used oral contraceptives. Among women who had received ambulatory care for pelvic inflammatory disease, no major differences emerged in fertility problems between oral contraceptive and intrauterine device users. However, among those who were hospitalized for pelvic inflammatory disease, oral contraceptive users were more likely to have fertility problems (55%) than intrauterine device users (45%).

In summary, our stratified analysis revealed the following findings: (1) About 23% of intrauterine device users and 17% of oral contraceptive users had had pelvic inflammatory disease. This difference was quite large among childless women (21% of intrauterine device users and 12% of oral contraceptive users), but it was smaller among women with children. (2) Women ever treated for pelvic inflammatory disease were far more likely to have fertility problems (44%) than those never treated for pelvic inflammatory disease (21%). This was true in both parity groups and for both oral contraceptive and intrauterine device users. (3) Women hospitalized for pelvic inflammatory disease were much more likely to have fertility problems (57%) than those treated in an ambulatory setting (39%). This was found in both parity groups and for both intrauterine device and oral contraceptive users, but the differences were not significant among the childless and among intrauterine device users.

Table VI. Fertility problems by contraceptive choice, parity, and hospitalized and ambulatory pelvic inflammatory disease: American women ages 15 to 44 years, 1982

	Self-reported pelvic inflammatory disease		
Contraceptive choice	*Ambulatory*	*Hospitalized*	*Any pelvic inflammatory disease*
Intrauterine device ever			
Parity			
All	35.1	45.1	37.8
0	*	*	*
1+	37.4	46.0	39.8
Oral contraceptive ever			
Parity			
All	36.6†	55.1	42.0
0	29.5	*	29.4
1+	39.5	61.9	46.6

*Number of cases <60.
†$p < 0.01$.

Multiple classification analysis

Controlling through multiple classification analysis for the simultaneous effects of age, pelvic inflammatory disease, parity, and contraceptive method choice on fertility problems (Table VII), three key findings emerge. First, independent of method choice, parity, and age, pelvic inflammatory disease, especially hospitalized pelvic inflammatory disease, greatly increases the likelihood of fertility problems. After controlling for other factors, 22% of women who were never treated for pelvic inflammatory disease had fertility problems compared with 37% of those who received ambulatory treatment and 53% of those who were hospitalized for pelvic inflammatory disease.

Second, after controlling for age, parity had no significant effect on the proportion with fertility problems; 24% of childless women and 25% of women with one or more children had fertility problems after controlling for age. Third, women who used oral contraceptives (but not intrauterine devices) were significantly more likely to have fertility problems (26%) than were women who ever used intrauterine devices (17%). Fourth, women who had never used a method were substantially more likely to have fertility problems (42%) than any of the other contraceptive method groups. Thus even after adjustment, although oral contraceptive users were afforded a protective effect from fertility problems compared with using no method at all, they were at increased risk compared with intrauterine device users.

The components of fertility problems reveal that surgical sterilization for noncontraceptive reasons is at least twice as common among women who had pelvic inflammatory disease than among those who did not

Table VII. Multiple classification analysis: Percentage of women 15 to 44 years of age with fertility problems by pelvic inflammatory disease, parity, and method: United States, 1982

	Observed	*Adjusted**	*Weighted N (thousands)*
Total	25	—	37,242
Pelvic inflammatory disease			
Never treated	22†	22†	31,584
Ambulatory	39‡	37§	3,952
Hospitalized	58	53	1,705
Parity			
Childless	16†	24‖	15,198
1 or more	31	25	22,044
Method			
Intrauterine device	26‖	17‡	6,272
Pill (but not intrauterine device)	24	26	22,261
Any except pill or intrauterine device	21†	24‡	6,291
Never used any method	40	42	2,418

*Adjusted for the other variables in the table, plus single years of age.

†$p < 0.001$.

‡$p < 0.01$.

§$p < 0.05$.

‖$p > 0.05$.

Table VIII. Contraceptive choice, pelvic inflammatory disease history, and resultant fertility status: American women ages 15 to 44 years, 1982

Ever used oral contraceptives (%)		
Pelvic inflammatory disease (16.8)		
No problems	58.0	
Infertile	1.5	19.0
Impaired	17.5	
Surgical sterilization—noncontraceptive*	22.9	
	100.0	
No pelvic inflammatory disease (83.2)		
No problems	79.5	
Infertile	1.6	11.6
Impaired	10.0	
Surgical sterilization—noncontraceptive	8.9	
	100.0	
Ever used intrauterine device (%)		
Pelvic inflammatory disease (23.3)		
No problems	62.2	
Infertile	0.7	15.2
Impaired	14.5	
Surgical sterilization—noncontraceptive	22.6	
	100.0	
No pelvic inflammatory disease (76.7)		
No problems	78.7	
Infertile	2.6	10.9
Impaired	8.3	
Surgical sterilization—noncontraceptive	10.5	
	100.0	

*Surgical sterilization not for contraceptive reasons.

(Table VIII). For example, among oral contraceptive users 23% of those who had pelvic inflammatory disease were surgically sterilized for noncontraceptive reasons compared with only 9% of those who had not. Infertility and impaired fecundity combined are also higher among the pelvic inflammatory disease group. Although the proportion of women who had pelvic inflammatory disease and are surgically sterilized is almost identical across contraceptive choice categories, the proportion of women who had pelvic inflammatory disease and are categorized as infertile or having impaired fecundity is higher among oral contraceptive users than among intrauterine device users.

Comment

Our analysis of the National Survey of Family Growth data provides national estimates of the prevalence of pelvic inflammatory disease and fertility problems among reproductive-age American women who have ever used oral contraceptives and intrauterine devices. Our findings of higher rates of pelvic inflammatory disease among intrauterine device users than oral contraceptive users generally agree with conclusions from multiple epidemiologic studies.

Two limitations of our particular data set must be acknowledged. First, we measured pelvic inflammatory disease through self-reports of women rather than by clinical diagnosis or laparoscopic findings. Even clinical factors alone are unreliable predictors of pelvic inflammatory disease.[10] Furthermore, patient factors such as intentional nonreporting, incomplete recall, and unawareness of the diagnosis could dilute the accuracy of these data. Second, the definition of fertility problems available from the National Survey of Family Growth is quite different from that used in clinical studies. Because all causes of infertility are included, we cannot differentiate that caused by tubal factors from other types. The effect of these two limitations would tend to blur any true relationships; thus our finding of consistent differences strengthens their validity.

Two main values of the National Survey of Family Growth are: (1) It provides population-based estimates of fertility problems and thus includes a high percentage of women who have never sought health care for such problems. These women would not be included in investigations of clinical infertility that are thus limited in the generalizability of their results, and (2) it allows for cross-sectional analysis of the interrelated factors of contraceptive choice, self-reported pelvic inflammatory disease, and fertility problems among a national sample of American women of reproductive age. As such, it adds a new dimension to our knowledge in this complex field.

One unexpected finding was that fertility problems were more frequent among oral contraceptive users.

This, we believe, could reflect the selection of women with proved fertility for intrauterine device use. For almost a decade before 1982, the intrauterine device was not recommended for nulliparous women.[12] Thus our data suggest that physicians may have been understandably reluctant to prescribe intrauterine devices to nulliparous women and those with fertility problems and perhaps prescribed the pill because of its hypothesized protective effect.

Oral contraceptive users who reported having had pelvic inflammatory disease were more likely to have fertility problems than were intrauterine device users who had pelvic inflammatory disease. This is consistent with the hypothesis that oral contraceptives may be associated with chlamydial pelvic inflammatory disease,[5] which is more difficult to recognize, diagnose, and treat in a timely fashion than is gonococcal pelvic inflammatory disease.[13, 14] By remaining silent and/or incompletely treated, chlamydial infections can smolder and lead to eventual fertility problems.

This hypothesis is also consistent with other findings from our analysis. First, the difference in fertility problems between women who were hospitalized for pelvic inflammatory disease and those who received ambulatory care is greater among users of oral contraceptives than among intrauterine device users. If hospitalization reflects severity of pelvic inflammatory disease, then this finding indicates that fertility problems among oral contraceptive users with more severe pelvic inflammatory disease are more common than among intrauterine device users with severe pelvic inflammatory disease. Second, the relationship between pelvic inflammatory disease and fertility problems may be more apparent among women hospitalized for pelvic inflammatory disease than among the ambulatory pelvic inflammatory disease group, since pelvic inflammatory disease can be more accurately diagnosed when the patient is hospitalized and less misclassification occurs.

Although most of our findings are consistent with hypothesized relationships among oral contraceptive use, chlamydial pelvic inflammatory disease, and eventual emergence of fertility problems, they do not provide direct support for these etiologic hypotheses. Given the limitations of our data set, we cannot rule out the possibility that women with fertility problems were somehow selected for oral contraceptive use. It is not implausible that some women with more severe or recurrent pelvic inflammatory disease were given oral contraceptives for therapeutic or preventive purposes.

In conclusion, our findings confirm the importance of contraceptive choice on pelvic inflammatory disease and fertility problems among reproductive-age American women. The adverse consequences of sexual intercourse, unplanned pregnancy and sexually transmitted infections, might be reduced if more attention was shown for common and primary prevention approaches. Barrier methods of contraception may be indicated for those women who want protection against both sexually transmitted diseases and unplanned pregnancy.

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REFERENCES

1. Lee NC, Rubin GL, Ory HW, Burkman RT. Type of intrauterine device and the risk of pelvic inflammatory disease. Obstet Gynecol 1983;62:1-6.
2. Daling JR, Weiss NS, Metch DJ, et al. Primary tubal infertility in relation to the use of an intrauterine device. N Engl J Med 1985;312:937-41.
3. Cramer DW, Schiff J, Schoenbaum SC, et al. Tubal infertility and the intrauterine device. N Engl J Med 1985;312:941-7.
4. Senanayake P, Kramer DG. Contraception and the etiology of pelvic inflammatory disease: new perspectives. AM J OBSTET GYNECOL 1980;138:852-60.
5. Washington AE, Gove S, Schachter J, Sweet R. Oral contraceptives, *Chlamydia trachomatis* infection, and pelvic inflammatory disease. A word of caution about protection. JAMA 1985;253:2246-50.
6. Harrison RH, Costin M, Meder J, et al. Cervical *Chlamydia trachomatis* infection in university women: relationship to history, contraception, ectopy and cervicitis. AM J OBSTET GYNECOL 1985;153:244-51.
7. Bachrach CA. Contraceptive practice among American women 1973-1982. Fam Plann Perspect 1984;16:253-9.
8. Washington AE, Cates W, Zaidi AA. Hospitalizations for pelvic inflammatory disease: epidemiology and trends in the United States, 1975-1981. JAMA 1984;251:2529-33.
9. Mosher WD. Reproductive impairments in the United States, 1965-1982. Demography 1985;22:415-30.
10. Aral SO, Mosher WD, Cates Jr W. Self-reported pelvic inflammatory disease in the U.S.: a common occurrence. Am J Public Health 1985;75:1216-9.
11. Bachrach CA, Horn MC, Mosher WD, et al. National Survey of Family Growth, Cycle III: sample design, weighting, and variance estimation. Vital Health Stat 1985; 98:1-22.
12. Westrom L, Bergtsson LP, Mardh PA. The risk of pelvic inflammatory disease in women using intrauterine contraceptive devices as compared to nonusers. Lancet 1976;2:221-7.
13. Thompson SE, Brooks C, Eschenbach DA, et al. High failure rates in outpatient treatment of salpingitis with either tetracycline alone or penicillin/ampicillin combination. AM J OBSTET GYNECOL 1985;152:635-41.
14. Sweet RL, Schacter J, Robbie MO. Failure of beta-lactam antibiotics to eradicate *Chlamydia trachomatis* in the endometrium despite apparent clinical cure of acute salpingitis. JAMA 1983;250:2641-5.

The use and limitations of endocervical Gram stains and mucopurulent cervicitis as predictors for *Chlamydia trachomatis* in female adolescents

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We evaluated the presence of polymorphonuclear cells on endocervical Gram stains and clinical indices of cervicitis as predictors of *Chlamydia trachomatis* infection in 193 sexually active female adolescents; 148 (77%) subjects had no complaints. *C. trachomatis* was isolated from 34 (18%) subjects. The incidence of *C. trachomatis* differed by race: it was isolated from 30% of black subjects and 7% of nonblack subjects. Polymorphonuclear cells on Gram stains were found to be associated with the presence of *C. trachomatis* ($\chi^2 = 33.2$, $p < 0.0001$). The sensitivity, specificity, and positive predictive value for polymorphonuclear cells to predict the presence of *C. trachomatis* were 91% (31/34), 65% (103/159), and 36% (31/87), respectively. When examined by ethnicity, polymorphonuclear cells remained significantly associated with *C. trachomatis* for black subjects ($\chi^2 = 24.3$, $p < 0.001$) but not for nonblack subjects ($\chi^2 = 2.49$, $p < 0.1$). Mucopurulent discharge, friability, or erythema-edema of ectopy were not reliable indicators for *C. trachomatis*. Endocervical Gram stains alone are useful to identify populations at risk for *C. trachomatis* infection. Caution must be advised when interpreting data from different population groups because of the variability in *C. trachomatis* prevalence. (AM J OBSTET GYNECOL 1987;157:65-71.)

Key words: Mucopurulent cervicitis, adolescents, *Chlamydia trachomatis*

Chlamydia trachomatis infects approximately 4 million men, women, and infants each year. The highest age-related prevalence for *C. trachomatis* occurs among female adolescents.[1-4] The diagnosis of chlamydial infection is imperative if its associated morbidities such as salpingitis, infertility, ectopic pregnancy, neonatal pneumonia, and conjunctivitis and possibly cervical dysplasia are to be prevented.[5-8] However, the identification of chlamydial genital infections has met obstacles from both a laboratory and a clinical perspective. Currently, definitive diagnosis of chlamydia requires tissue culture isolation, which is costly and not readily available in many clinical settings. Recently, rapid method diagnostic techniques have been introduced. However, the performance of these tests has not been adequately assessed in a variety of populations.[9] Cost and availability of both the tissue culture and rapid diagnostic tests preclude their widespread use as screening tools for chlamydia.

Symptoms of lower genital tract infections tested as potential diagnostic indices for chlamydial endocervicitis have been found to be nonspecific. The use of symptomatic predictors is even more disappointing, since chlamydial infections are asymptomatic in the majority of infected women.

Many authors[3, 6, 10, 11] have reported relationships between the presence of cervicitis on examination and the isolation of chlamydia. The inconsistent nomenclature for cervicitis, such as follicular cervicitis, mucopurulent cervicitis, or cervical erosion, demonstrates the lack of definition standards. Clinical definitions for cervicitis vary among studies and have included combinations of gross appearance and laboratory findings.[3, 6, 10, 11] Most clinical signs of chlamydial infection on gross appearance are not objective. Also, populations studied have been largely from sexually transmitted disease clinics. Cervicitis has not been adequately evaluated in a non-sexually transmitted disease clinic population.

The aims of this study were to evaluate the following in a sexually active adolescent female population in a

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Table I. Performance of identification of polymorphonuclear cells on gram stains of endocervical secretion to predict *C. trachomatis* infection in all patients and by race

Polymorphonuclear cells on Gram stain	*Overall*		*Black*		*Non-black*	
	Positive	*Negative*	*Positive*	*Negative*	*Positive*	*Negative*
≥5	31	56	26	23	5	34
<5	3	103	1	39	2	66
Sensitivity (%)	91		96		71	
Specificity (%)	65		63		66	
Positive predictive value (%)	36		53		13	
Negative predictive value (%)	98		98		97	
Chlamydial incidence (%)	18		30		7	
p	<0.0001*		<0.001		<0.1	

*Association remained significant while controlling for *N. gonorrhoeae* infection, $\chi^2 = 33.2$ (with Yates' correction, $p < 0.0001$).

non–sexually transmitted disease clinic setting: (1) the predictive value of polymorphonuclear cells on endocervical Gram stain for current endocervical *C. trachomatis* infections in symptomatic and asymptomatic populations and (2) the predictive value of cervicitis on gross appearance for *C. trachomatis* infections.

Methods

Subjects. Sexually active adolescents (N = 193) aged 12 to 20 years, who were seen at the University of California, San Francisco Adolescent Clinics between October 1983 and December 1984 served as subjects. All patients having a pelvic examination for any reason were entered into the study. Patients were excluded if they had a spontaneous or therapeutic abortion within 30 days, were pregnant, or had taken antibiotics within 21 days. Patients were entered into the study only once. Reasons for visit were divided into two categories: lower genital tract–related and nonrelated complaints. Lower genital tract–related complaints were described as vaginal discharge, possible vaginal infection, and frequency or burning or dysuria.

Study design. History, including age, race, contraceptive method, age at onset of sexual activity, reason for visit, symptoms, and date of last menstrual period, was recorded on standardized forms. Adolescent medicine fellows and trained house staff members performed physical examinations; specimens were collected by standard protocol. Clinical cervicitis was designated if any one of the following was noted on examination: (1) endocervical mucopurulent discharge (yellow-green discharge from the os on a white-tipped swab), (2) cervical erythema or edema of ectopy (as noted by the unaided eye), and (3) cervical friability (bleeding after manipulation of the endocervix with one swab). Data on endocervical discharge were missing on six subjects. Sixteen subjects had significant endocervical bleeding (caused by either gross friability or intermenstrual bleeding). Endocervical discharge was reported as missing for those subjects. Information on cervical friability or erythema-edema was not recorded clearly in 37 subjects. Subjects with any missing data were excluded when the analysis performed included all parameters.

All microbiologic cultures and endocervical Gram stains were collected according to the standardized protocol used in the clinic. After the cervix was cleaned of vaginal contamination with a large cotton swab, endocervical cells for Papanicolaou smear were collected. A Dacron swab was rotated 360 degrees for at least three rotations, removed, and examined for mucopurulent discharge. The swab was then rotated onto a glass slide 0 to 180 degrees for the Papanicolaou smear, and then placed on a slide for Gram stain. After the Papanicolaou smear was completed, a second swab was introduced into the endocervix and placed into charcoal media for transport to the laboratory for isolation of *Neisseria gonorrhoeae* by standard technique.

Two endocervical specimens for *C. trachomatis* cultures were obtained with a wooden tapered spatula. Transport and isolation techniques have been described in detail elsewhere.[2] Vaginal secretions were then collected from all patients for pH value and microscopic examination with 10% potassium hydroxide and normal saline solution on a glass slide.

The diagnosis of bacterial vaginosis was made according to modified criteria by Amsel et al.[12] At the time of the study the presence of a gray homogeneous discharge was not documented. Therefore the diagnosis of bacterial vaginosis was modified to include at least two of the three following criteria: (1) pH ≥ 5.0, (2) ≥20% clue cells on wet mount preparation with normal saline solution, or (3) fishy odor detected after 10% potassium hydroxide was added to the vaginal secretion on a glass slide. Yeast and *Trichomonas vaginalis* were diagnosed if found on either wet mounts or Papanicolaou smear. Herpes simplex viral cultures were not performed routinely because of cost and low incidence in our population (<0.5%).

Gram stains were reviewed without knowledge of patient identification and diagnosis. Endocervical smears were heat fixed and stained with rapid method Gram stain technique. The number of polymorphonuclear cells at a magnification of × 1000, with oil immersion, on five nonadjacent fields was determined, averaged, and categorized as follows: (1) 0 to 4, (2) 5 to 9, (3) 10 to 14, or (4) ≥15. Cervical mucus was found to have been applied in a patchy distribution on most slides. Thus areas selected for evaluaiton on the Gram stain slide included those with a monolayer distribution of polymorphonuclear cells. Forty-four Gram stains were chosen randomly to be reviewed by a second observer to measure interobserver reliability. The second observer categorized the number of polymorphonuclear cells into <5, ≥5, ≥10, and ≥15 polymorphonuclear cells per high-powered field. There was 80% agreement on Gram stain results.

Statistical analysis. χ^2 Analyses were used to test the significance of the association between relevant variables. Logistic regression analyses were used to determine independent predictors of *C. trachomatis* endocervical infections. p Values were based on two-tailed tests. When multiple tests were performed, the p value was set at the <0.01 level.

Results

Demographic data. The mean age of subjects was 17.3 years (SD ± 1.66); 89 (45%) were black, 65 (34%) were white, and 39 (21%) had other racial backgrounds. The mean age at onset of sexual activity was 14.8 (SD ± 1.53 years), and the mean number of lifetime sexual partners was 4.5 (SD ± 8.7). Within 60 days before examination, 67 (35%) subjects had used oral contraception, 62 (32%) used barrier methods, and 64 (33%) used withdrawal or no method. Forty-five (23%) subjects initially had a chief complaint related to lower genital tract infections.

Incidence. *C. trachomatis* was isolated in 34/193 (18%) subjects. *N. gonorrhoeae* was isolated in 16 (8%) subjects and *T. vaginalis* in 6 (3.1%) subjects. Bacterial vaginosis was identified in 12 (6.2%) subjects and yeast vaginitis in 21 (11%) subjects. Seven (44%) subjects with *N. gonorrhoeae* had concomitant *C. trachomatis* infection.

Univariate analyses

Correlates of C. trachomatis *infection*

Age and race. There was a trend between younger age and isolation of *C. trachomatis.* Thirty-one percent of subjects younger then age 16 years were *C. trachomatis* positive, compared with 17% of subjects aged 16 to 17 years and 10% of subjects aged 18 years or older (χ^2 = 6.28, $p < 0.04$).

C. trachomatis infection also varied as a function of race. Among black subjects, the incidence was 30%, and among nonblack subjects, the rate was 7% (χ^2 = 19.49, $p < 0.0001$). (Because the chlamydial incidence rates of white subjects and others were similar, they were grouped into one population.) There was no difference in the mean number of lifetime partners between black subjects and others (five versus four, respectively).

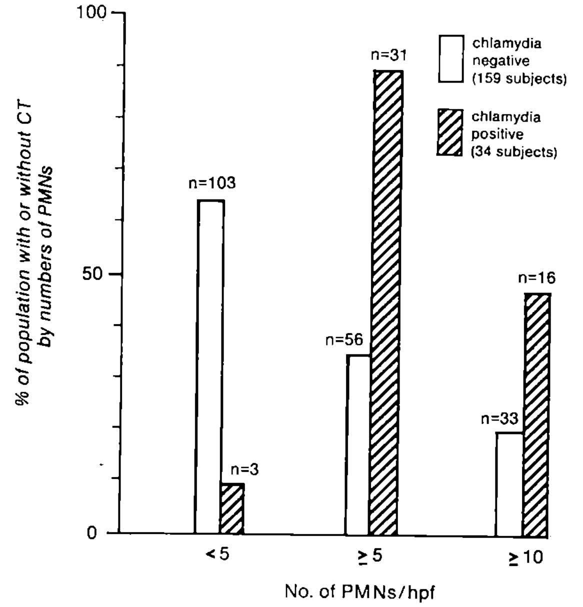

Fig. 1. Histogram of polymorphonuclear cells *(Pmns)* per high-powered field *(hpf)* from subjects with and without endocervical chlamydia. n = Number of subjects in corresponding groups by polymorphonuclear cells.

Factors that were not associated with *C. trachomatis* infection included date of menstrual cycle, age at onset of sexual activity, reason for visit, and lower genital tract symptoms. Of 34 subjects with chlamydia, only 11 (38%) had lower genital tract–related chief complaints. There was no significant difference in method of birth control in subjects with or without *C. trachomatis* infection. Thirty-five percent in either group used no method. Twenty-six percent and 32% used oral contraceptives, and 38% and 32% used barrier methods in the *C. trachomatis* positive and negative groups, respectively.

Gram stain. The presence of polymorphonuclear cells on Gram stains (≥five polymorphonuclear cells/high-powered field) was significantly associated with chlamydial infection as shown in Table I. Gram stains with threshold values of ≥10 or ≥15 polymorphonuclear cells per high-powered field were not better predictors of *C. trachomatis* than Gram stains with threshold values of ≥five polymorphonuclear cells per high-powered field. Sensitivity and specificity at ≥10 polymorphonuclear cells per high-powered field were 47% (16/34) and 79% (126/159), respectively, and at ≥15 polymorphonuclear cells per high-powered field were 38% (13/34) and 89% (142/159), respectively (Fig. 1). When examined by race, the association of

Table II. Association between signs of endocervical infection and *C. trachomatis*

Clinical finding	C. trachomatis (*n*)		χ^2
	Positive	*Negative*	
Mucopus*			
Positive	10	21	3.99†
Negative	21	119	
Erythema and/or edema of ectopy‡			
Positive	7	8	9.96§
Negative	17	124	
Friability‡			
Positive	9	23	4.46†
Negative	14	140	
Cervicitis‖			
Positive	12	27	7.14¶
Negative	11	93	

*Six subjects had missing data, and 16 subjects were excluded from analyses because of presence of bleeding.

†$p < 0.05$.

‡Incomplete data for 37 subjects.

§$p < 0.002$.

‖Cervicitis is defined by the presence of any one of the following: endocervical mucopurulent discharge, friability, or ectopic erythema-edema. Subjects with any missing data or endocervical bleeding were excluded.

¶$p < 0.0001$.

Gram stain and *C. trachomatis* was significant in black subjects ($\chi^2 = 24.3$, $p < 0.0001$) but became insignificant in non-black subjects ($\chi^2 = 2.49$, $p < 0.1$) (Table I).

Of 87 subjects with ≥five polymorphonuclear cells per high-powered field on Gram stain, only eight had a positive culture for *N. gonorrhoeae* ($\chi^2 = 0.03$, $p < 0.9$). Of those with *N. gonorrhoeae*, 8/16 (50%) had ≥five polymorphonuclear cells per high-powered field. Polymorphonuclear cells on Gram stains were also not significantly associated with the presence of *T. vaginalis*, bacterial vaginosis, yeast vaginitis, ectopy, reason for visit, or race. Neither contraceptive method nor last menstrual period was related to the presence of polymorphonuclear cells. The number of polymorphonuclear cells per high-powered field found was equally distributed throughout the menstrual cycle in women with and without *C. trachomatis* infection.

Clinical findings. Analyses showed a significant association between *C. trachomatis* and the presence of erythema or edema of the ectopy on examination ($p < 0.002$) (Tables II and III). Mucopurulent endocervical discharge and friability were not associated with *C. trachomatis* infection, although trends toward positive associations were found (p values < 0.05) (Tables II and III). Among subjects with endocervical mucopurulent discharge, 26 of 31 (84%) had ≥five polymorphonuclear cells per high-powered field. The associations found between mucopurulent discharge, erythema or edema, or friability and *C. trachomatis* were similar when examined by race. Cervicitis (the presence of any one of the following: endocervical mucopurulent discharge, friability, or ectopic erythema-edema) was found to be significantly related to the presence of chlamydial infection (Tables II and III).

Multivariate analyses. Although the above analyses illustrate how individual factors relate to *C. trachomatis*, they do not take into account the intercorrelations among these variables. To examine the unique variance accounted for by each factor, a series of hierarchical multiple logistic regression analyses was performed.

In the first analysis, polymorphonuclear cells on Gram stain, mucopurulent discharge, friability, and ectopic erythema or edema were entered into the equation. Polymorphonuclear cells were the only significant predictor of *C. trachomatis* ($p < 0.001$). Mucopurulent discharge, erythema-edema, and friability were insignificant ($p < 0.5$, $p < 0.2$, $p < 0.3$, respectively). The addition of symptoms (abnormal discharge or bleeding) into the equation was not significant.

Comment

Identification of polymorphonuclear cells (≥five per high-powered field) on Gram stains of endocervical secretions is a useful screening tool in predicting endocervical chlamydial infection. In our population the sensitivity of this indicator is 91% (31/34), the specificity is 65% (103/159), and the positive predictive value is 36% (31/87). With this screening tool, 64% of those adolescents with ≥five polymorphonuclear cells per high-powered field would be incorrectly identified as being *C. trachomatis* positive. However, only 9% of chlamydial infections would remain undetected. These findings in Gram stains are similar to previous works.[3, 6, 10, 11] In contrast to other studies and to the recommendations of the Centers for Disease Control, our study suggests that a cutoff value of ≥five but not ≥10 polymorphonuclear cells per high-powered field is more effective in determining the presence of chlamydial endocervical infection in adolescents. The sensitivity at ≥five polymorphonuclear cells per high-powered field was significantly higher than at ≥10 polymorphonuclear cells per high-powered field, 91% and 44%, respectively (Fig. 1).

The performance of the Gram stain test to predict *C. trachomatis* infections varied by race. The test's performance was significantly better among black subjects. In nonblack subjects, polymorphonuclear cells were not predictive of *C. trachomatis* infections. Among black subjects who had a *C. trachomatis* incidence of 30%, the test yielded a 53% predictive value. In nonblack subjects with an incidence of 7%, the positive predictive value

Table III. Sensitivity, specificity, and predictive values for clinical findings and isolation of *C. trachomatis*

	Polymorphonuclear cells	*Mucopurulent discharge*	*Erythema-edema*	*Friability*	*Cervicitis**
Sensitivity (%)	91	32	29	39	52
Specificity (%)	65	85	94	83	77
Positive predictive value (%)	36	32	47	28	31
Negative predictive value (%)	98	85	88	89	89

*See Table II for definition.

was only 13%. The variation in significance of the Gram stain to predict *C. trachomatis* by race may be explained in part by these marked differences in the incidence of *C. trachomatis*. The diagnosis of mucopurulent discharge as a visible clinical sign was not associated with *C. trachomatis* infection in our population. Mucopurulent discharge predicted chlamydial infection in 10/31 (32%) subjects and was found in only 10/31 (32%) subjects with *C. trachomatis*. The recent recommendation by the Centers for Disease Control to treat all women with the clinical sign of mucopurulent endocervical discharge (definied as a yellow endocervical discharge noted on a white-tipped swab) was based on a study that reported that mucopurulent discharge had a positive predictive value for *C. trachomatis* of 54%.[1, 11] This study was performed in a sexually transmitted disease clinic. The incidence of chlamydia was high (20%), and 95% of the subjects were symptomatic for genitourinary disease or were known contacts of subjects with sexually transmitted disease. The poor performance of mucopurulent discharge, with a sensitivity of only 32%, as a predictor of *C. trachomatis* in the current study may be the result of the high proportion of asymptomatic subjects screened in our non–sexually transmitted disease clinic. Multiple clinicians making subjective determinations of the presence of yellow color on a endocervical swab also may have influenced the results. However, our site is similar to most busy clinical settings. Therefore the usefulness of endocervical mucopurulent discharge on a swab outside of a controlled clinical environment is questionable. Another factor that may have influenced these findings was the low incidence ($<1\%$) of endometritis and salpingitis in our population. It has been suggested that mucopurulent discharge is a possible clinical sign of endometritis and salpingitis.[6] This low incidence in our adolescents is unexplained; however, our vigorous screening program including persistent partner identification and treatment, for *C. trachomatis* or *N. gonorrhoeae* may have contributed.

In the study by Brunham et al.,[11] neither friability nor ectopic erythema or edema was significantly associated with *C. trachomatis*. Despite these findings the Centers for Disease Control recommends their presence for identification of *C. trachomatis*.[1] Our findings revealed that, in addition to mucopurulent discharge, friability of the cervix was also found to be only weakly associated with *C. trachomatis* infection. The sign of visible ectopic erythema or edema was associated strongly with *C. trachomatis* infection ($p < 0.002$). However, its sensitivity was quite low (29%). Although mucopurulent discharge, friability, and ectopic erythema-edema showed univariate associations with *C. trachomatis*, multivariate analysis showed that polymorphonuclear cells on Gram stain were the only significant predictor of *C. trachomatis* infection. Thus, when the intercorrelations between clinical diagnostic indices were controlled for, the presence of polymorphonuclear cells on Gram stains remained the only significant factor associated with *C. trachomatis*. This suggests that the univariate relationships found between erythema-edema, friability, and mucopurulent discharge and *C. trachomatis* were primarily the result of the relationship between these three factors and polymorphonuclear cells.

Although intermenstrual bleeding, vaginal discharge, and abdominal pain have been associated with endocervical *C. trachomatis* infection by others,[2-4] we found no such associations. When the presence of symptoms was analyzed with the number of polymorphonuclear cells, these symptoms did not strengthen the relationship between the Gram stain and *C. trachomatis* infection. Such findings illustrate the importance of screening women for *C. trachomatis* infection with and without genitourinary symptoms, whether culture or Gram stains are used as the screening tools.

The importance of identifying *C. trachomatis* among high-risk groups has been well documented.[2-8] Identification of *C. trachomatis* by tissue culture is sensitive, although expensive, and requires sophisticated laboratory support that is not widely available. Studies have demonstrated that antibody tests used for detection of cervical *C. trachomatis* infection in several clinical situations are less sensitive than tissue culture.[9, 13] In our previous work the sensitivity of the *C. trachomatis* monoclonal antibody test in a female adolescent population with culture as a gold standard was only 61%, with a specificity of 97%.[13]

Thus, in conclusion, in its attempt to control the chlamydial epidemic, the Centers for Disease Control acknowledges the obstacles discussed above but has made recommendations to treat all women identified with mucopurulent cervicitis for *C. trachomatis* infection.*

In our study we have found that the presence of polymorphonuclear cells on Gram stain (≥five/high-powered field) is a useful screening tool that offers immediate results, requires little or no additional training for clinical personnel, is inexpensive, and is widely available. This Gram stain test has acceptable sensitivity, specificity, and positive predictive value to support its use as a screening device for endocervical chlamydial infection in adolescents for whom tissue culture and rapid method diagnostic tests are not feasible or available. In comparison with the severe outcomes of *C. trachomatis* infections such as acute salpingitis or ectopic pregnancies, a low-cost test with a predictive value of only 36% is not only acceptable but cost effective.[14] Traditionally, the presence of polymorphonuclear cells in the absence of gram-negative intracellular diplococci on the Gram stain of the urethral smear has been used in male subjects to predict chlamydial infections. The predictive value for *C. trachomatis* in men with positive smears is 30% to 60%.[15] Although the Gram stain predictability for chlamydial infection is greater in men, it still remains a useful indicator of chlamydial infection in women. However, the test's accuracy may be dependent on the population's *C. trachomatis* incidence. Incidence of *C. trachomatis* should be determined in the population before the use of the Gram stain as a reliable test for *C. trachomatis*. The Gram stain may also serve the purpose of identifying a high-risk population that would benefit from testing for *C. trachomatis*. This may be especially useful if the necessary resources are available yet limited.

Adolescents may represent a unique population. Puberty is a transitional time when the cervix undergoes maturative changes defined as squamous metaplasia. Mild inflammatory responses may occur because of such events. This transitional event accompanied by inflammation may be susceptible to *C. trachomatis* infections rather than the inflammatory response being caused by *C. trachomatis* infection. This normal response to squamous metaplasia may explain the presence of cervicitis in 50% of our population.

Contrary to current recommendations, other clinical parameters were not reliable tests for chlamydial infection. It must be kept in mind that at best the predictive value of cervical changes for *C. trachomatis* is <50%. This inflammatory response seen in the cervix may be the early manifestation of cervical disruption leading to acute salpingitis or cervical dysplasia. Alarmingly, younger women appear to be at a higher risk for salpingitis than older women, and the mean age for cervical dysplasia appears to be decreasing.[8, 16] Thus future studies should include the search for other pathogens capable of creating an inflammatory reaction. This low predictive value for *C. trachomatis* also expresses the need for follow-up examinations after presumptive treatment, recognizing that other pathogens may be responsible for the cervical changes resistant to tetracycline.

REFERENCES

1. Division of Sexually Transmitted Diseases, Center for Prevention Services, Centers for Disease Control. *Chlamydia trachomatis* infections: policy guidelines for prevention and control. MMWR 1985;34:30.
2. Shafer MA, Beck A, Blain B, et al. *Chlamydia trachomatis:* important relationships to race, contraceptive use, lower genital tract infection and Papanicolaou smears. J Pediatr 1984;104:141-6.
3. Saltz GR, Linneman CC Jr, Brookman RR, Raul JL. *Chlamydia trachomatis* cervical infections in female adolescents. J Pediatr 1981;98:981.
4. Schachter J, Stoner E, Moncada J. Screening for chlamydial infections in women attending family planning clinics. West J Med 1983;138:35.
5. Hare MJ, Taylor-Robinson D, Cooper P. Evidence for an association between *Chlamydia trachomatis* and cervical intraepithelial neoplasia. Br J Obstet Gynaecol 1982;89:489.
6. Paavonen J, Brunham R, Kiviat N, et al. Clinical and histologic evidence of endometritis among women with cervicitis. In: Mardh P-A, Holmes KK, Oriel JD, Piot T, Schachter J, eds. Chlamydia infection. New York: Elsevier, 1982:163.
7. Honnaess H, Dalaker K, Anestad G, Mardh D, Kuice G, Bergan L. Pelvic inflammatory disease: etiologic studies with emphasis on Chlamydial infection. Obstet Gynecol 1982;59:550.
8. Sweet RL. Chlamydia salpingitis and infertility. Fertil Steril 1982;38:530.
9. Lipkin E, Moncada J, Shafer MA, Wilson T, Schachter J. Comparison of monoclonal antibody staining and culture in diagnosing cervical chlamydial infection. J Clin Microbiol 1986;23:114.
10. Paavonen J, Vesterinen E. *Chlamydia trachomatis* in cervicitis and urethritis in women. J Infect Dis 1982;32:45.
11. Brunham R, Paavonen J, Stevens C, et al. Mucopurulent cervicitis—the ignored counterpart in women of urethritis in men. N Engl J Med 1984;311:3.
12. Amsel R, Totten PA, Spiegel CA, et al. Nonspecific vaginitis: diagnostic criteria and microbial and epidemiologic associations. Am J Med 1983;74:14.
13. Shafer MA, Vaughan E, Lipkin ES, Moscicki B, Schachter J. Evaluation of fluorescein-conjugated monoclonal antibody test to detect *Chlamydial tracomatis* endocervical infections in adolescent girls. J Pediatr 1986;108:779.
14. Nettleman MD, Jones RB, Roberts SD, et al. Cost-

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*Criteria for presumptive diagnosis include (1) mucopurulent secretion from the endocervix, which is usually yellow or green when viewed on a white cotton-tipped swab (positive swab test); (2) ≥10 polymorphonuclear leukocytes per microscopic oil immersion field (magnification, × 1000) in a Gram-stained smear of endocervical secretions; and (3) cervicitis, determined by cervical friability (bleeding when the first swab culture is taken) and/or erythema or edema within a zone of cervical ectopy.[1]

effectiveness of culturing for *Chlamydia trachomatis*. Ann Intern Med 1986;105:189.

15. Stamm WE, Koutsky LA, Benedetti JK, Jourdan JL, Brunham RC, Holmes KK. *Chlamydia trachomatis* urethral infections in men. Prevalence, risk factors and clinical manifestations. Ann Intern Med 1984;100:47.
16. Cook GA, Draper GJ. Trends in cervical cancer and carcinoma in situ in Great Britain. Br J Cancer 1984;50:367-75.

The value of curettage in the diagnosis of ectopic pregnancy

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The charts of all patients hospitalized between 1978 and 1983 with proved ectopic pregnancies at Northwestern Memorial Hospital, Chicago, and Meir Hospital, Kfar-Sava, Israel, were reviewed. Eighty-four patients with ectopic pregnancies had endometrial tissue available for histologic analyses. Review of the endometrial curettings revealed that the most common endometrium associated with ectopic pregnancy was secretory (39.4%). Proliferative endometrium present 19% of the time was as common a finding as Arias-Stella phenomenon. This study shows that any type of endometrium lacking trophoblasts may be associated with an ectopic pregnancy. The lack of decidual reaction or Arias-Stella phenomenon should not alone lower the clinician's index of suspicion. (AM J OBSTET GYNECOL 1987;157:71-2.)

Key words: Ectopic pregnancy, curettage, endometrium

The differentiation between ectopic pregnancies and complications of early intrauterine pregnancy continues to be a diagnostic dilemma for clinicians. When the continuation of the pregnancy is not desired or the pregnancy is considered nonviable, uterine curettage is usually performed. The pathologic diagnosis of the endometrial curettings may raise the first suspicion for an extrauterine pregnancy. The lack or presence of trophoblastic tissue is critical in distinguishing between intrauterine and extrauterine pregnancies. Clinicians have been trained to react to the presence of the Arias-Stella phenomenon or decidual reaction in the endometrium. The absence of these features may lead the clinician to a false diagnosis of complete abortion and away from a diagnosis of ectopic pregnancy.

This study was undertaken to investigate the distribution of the various types of endometrium in patients with ectopic pregnancies. A search of the literature over the past 25 years failed to reveal any study addressing this question.

Table I. Histologic diagnoses of 84 study patients

Histologic findings	*No. of patients*	%
Arias-Stella reaction	16	19.0
Decidual reaction	19	22.6
Secretory endometrium	33	39.4
Proliferative endometrium	16	19.0
Total	84	100.0

Material and methods

The medical records of all the patients with proved ectopic pregnancies who were hospitalized from 1978 to 1983 at Northwestern Memorial Hospital, Chicago, Illinois, and Meir Hospital, Kfar-Sava, Israel, were reviewed. Of these patients, 84 had endometrial tissue available for histologic analysis. The distribution of patients between Northwestern Memorial Hospital and Meir Hospital was about equal; the numbers were 40 and 44, respectively. In each hospital, about 15% of the patients with diagnosed ectopic pregnancies underwent curettage. The pathologic findings of these patients were recorded, and a statistical analysis was made of the type of endometrium present.

Results

The histologic diagnoses of the 84 patients included in this study are outlined in Table I. Only sixteen pa-

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tients (19%) had Arias-Stella phenomenon, and 19 (22.6%) had decidual reaction. Secretory endometrium was the most common pathologic diagnosis associated with ectopic pregnancy and was found in 33 patients (39.4%). Sixteen patients (19%) had proliferative endometrium.

Comment

Over the past 15 years there has been not only a threefold increase in the number of ectopic pregnancies, but the rate of ectopic pregnancies has also increased dramatically. Dorfman[1] reported that in 1970 there were 17,800 ectopic pregnancies, representing 0.45% of all pregnancies, but in 1980 this number had increased to 52,200, or 1% of all pregnancies. Some institutions report that one of every 84 pregnancies is ectopic. Despite the advent of ultrasonography, laparoscopy, and very sensitive quantitative tests for β-subunit of human chorionic gonadotropin, the risk of death from an ectopic pregnancy is 10 times that of a legal abortion. One of the major reasons why there are deaths from ectopic pregnancies is because of delays by the clinician either because of a lack of suspicion or an improper reassurance from the assessment of information available. Schneider et al.[2] reviewed 102 deaths attributable to ectopic pregnancy. Their study showed that patients with an ectopic pregnancy sought medical help for symptoms an average of 3.8 days before admission and treatment.

Different views exist about the value of endometrial curettage in the diagnosis of ectopic pregnancy. Most authors find curetting to be a valuable diagnostic tool.[3-5] The consensus of these authors indicates that endometrial curetting can be predictive of ectopic pregnancy in 40% to 60% of patients.[5-7] Keresztury[8] felt that when great care and attention were taken in analyzing curettings, a predictive diagnosis of an ectopic pregnancy could be made in 76% of patients.

In contrast, Novak and Woodruff[9] do not consider endometrial curetting diagnostically helpful. This is especially the case when bleeding occurred before curettage. In a recent publication, Gal and Stenning[10] analyzed tissue from elective abortions in Australia. They discuss two patients whose curettings showed no evidence of a recent pregnancy but who were later diagnosed as having tubal pregnancies. Histologic examination revealed secretory endometrium in the first patient and "feeble secretory activity" in the second patient.

In the present study only 19% of the patients had endometrium revealing Arias-Stella phenomenon and another 22.6% had decidual reaction. Thus by the use of curettage material alone, ectopic pregnancy would be suspected in 41.6% of the patients. Most patients (58.4%) had neither of the characteristic pathologic findings usually associated with an ectopic pregnancy. Surprisingly, 16 of the patients (19%) had proliferative endometrium, making this finding as common as Arias-Stella reaction. We consider this finding very important, since the finding of proliferative endometrium in curettings can lead the clinician to a diagnosis of complete abortion. The false assurance of this diagnosis could delay the true diagnosis until the patient has a more serious complication.

The type of endometrium found during curettage is dependent on whether hormonally active trophoblastic tissue is present and also on the presence or absence of significant uterine bleeding. After prolonged bleeding the usefulness of curettage becomes significantly less,[1-3, 6, 7] and the typical findings are stromal atrophy associated with regenerative foci of proliferative glands. Sixteen of the 84 patients in our study had curettings that showed only proliferative endometrium.

Our observation emphasizes the fact that an ectopic pregnancy may be associated with any histologic type of endometrium. An ectopic pregnancy should be suspected any time a patient with a positive human chorionic gonadotropin test has no trophoblasts in a curettage specimen. Neither the absence of decidual reaction or an Arias-Stella reaction nor the presence of proliferative endometrium should lower the clinician's level of suspicion about the potential presence of an ectopic pregnancy. When trophoblasts are absent, an ectopic pregnancy must be suspected. There is no value but potentially a real danger in attempting to evaluate the risk of an ectopic pregnancy as a function of the type of endometrium found with curettage.

REFERENCES

1. Dorfman SF. Ectopic pregnancy 'thinking ectopic,' key to diagnosis. Postgrad Medi 1984;76:65-8.
2. Schneider J, Berger C, Cattell C. Maternal mortality due to ectopic pregnancy—a review of 102 deaths. Obstet Gynecol 1977;49:557-61.
3. Beato M, Castaño-Almendral A, Beato W. Der historische Befund des Endometriums zur Differentialdiagnose der Extrauteringraviditaet. Geburtshilfe Frauenheilkd 1968; 28:355.
4. Overbeck L. Das Endometrium bei abgestorbener Tubargraviditaet. Virchows Arch Pathol Anat 1953;324:409.
5. Saavedra J, et al. Das Arias Stella phaenomen und sein diagnostischer Wert bei ektopen Schwangenschaft. Zentralbl Gynaecol 1975;97:1166-75.
6. Mackles A, Wolfe S, Pozner S. Cellular atypia in endometrial glands (Arias-Stella reaction) as an aid in the diagnosis of ectopic pregnancy. AM J OBSTET GYNECOL 1961;81:1209.
7. Schultze K, Schultze. Klinische Erfahrung bei 150 Faellen von Extrauteringraeviditaet. Zentralbl Gynaecol 1954; 76:988.
8. Keresztury S. Examination of curettage material for the diagnosis of ectopic pregnancy. Acta Morphol Hung 1976;24:359-67.
9. Novak E, Woodruff JD. Gynecologic and obstetric pathology. 7th ed. Philadelphia: WB Saunders, 1974:494-503.
10. Gal A, Stenning H. Pitfalls in the diagnosis of ectopic pregnancy. Med J Aust 1985;143:411-2.

Observations on the log human chorionic gonadotropin–time relationship in early pregnancy and its practical implications

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In this study, we examined whether the doubling time of human chorionic gonadotropin is different at different stages of early pregnancy and whether the use of multiple nomograms for doubling time of human chorionic gonadotropin rather than a single critical value can improve the diagnosis of ectopic pregnancy. Forty-four women with intrauterine pregnancies who had abdominal pain and/or vaginal bleeding but who did not abort their pregnancies and 44 women with surgically proved ectopic pregnancies were studied. No difference was found in the doubling time of human chorionic gonadotropin when women were classified according to recently published criteria (Pittaway DE, Reish RL, Wentz AC. Doubling times of human chorionic gonadotropin increase in early viable intrauterine pregnancies. AM J OBSTET GYNECOL 1985;152:299-302), and estimates of the doubling time of human chorionic gonadotropin were not influenced by the initial human chorionic gonadotropin values or the sampling interval used. In the human chorionic gonadotropin range of practical interest, multiple nomograms identified 26 of 33 (79%) women with ectopic pregnancy who had rising levels of human chorionic gonadotropin, whereas our previously reported criteria identified 28 of 37 (76%) cases. The false positive rate for each method was 9.7%. Seventeen (19%) of 88 patients could not be allotted to the human chorionic gonadotropin categories for which nomograms of the doubling time of human chorionic gonadotropin have been derived. We conclude that our previous recommendations for determining the rate of increase of human chorionic gonadotropin in serum from paired samples do not require revision at this time. (AM J OBSTET GYNECOL 1987;157:73-8.)

Key words: Chorionic gonadotropin, human; pregnancy, early; chorionic gonadotropin, doubling times; gonadotropin

It is generally accepted that the determination of the rate of increase of human chorionic gonadotropin (hCG) in serum is a clinically useful test that can spare some women an operative procedure for a presumptive diagnosis of ectopic pregnancy.[1, 2] The candidates for serial hCG testing are women who have abdominal pain and/or vaginal bleeding, who do not require immediate surgery on clinical grounds, who have positive hCG assay results, and who have nondiagnostic findings on culdocentesis and pelvic ultrasonography.[3, 4] As previously reported, nondiagnostic ultrasound findings occur only when serum hCG is below 6000 mIU/ml (first internal reference preparation), so that serial hCG determinations are indicated only if the initial serum hCG is below this level.[4, 5]

The rate of hCG increase in serum and its fiducial limits can be expressed in several equivalent ways (in terms of the slope of the log hCG-time curve, hCG doubling time, and the percentage increase in serum hCG over a given sampling interval) but requires an assumption about the nature of the serum hCG–time relationship. The fiducial limits originally described by Kadar et al.[3] were based on the assumption that this relationship is exponential, that is, that the log hCG-time curve is linear, since earlier investigators have found this to be the case during the first 6 weeks of amenorrhea,[6-8] which corresponds to an hCG level of approximately 6000 mIU/ml (first internal reference preparation).[9]

Recently, Pittaway et al.[10] presented evidence suggesting that the log hCG-time relationship in early pregnancy might be better described by a quadratic equation than by a linear one, and the authors suggested that the use of multiple nomograms for the doubling time of hCG, corresponding to different ranges of serum hCG, might improve the detection of ectopic pregnancies. Subsequently, they adduced evidence to support their suggestion by showing, with the use of receiver-operating characteristics curve analysis, that more abnormal pregnancies were indeed detected for a given false positive rate when multiple rather than single nomograms for doubling time of hCG were used.[11]

Unfortunately, however, it is difficult to extrapolate their data to the common practical situations in which the differential diagnosis of ectopic pregnancy presents

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Table I. hCG doubling times, initial hCG values, and sampling intervals between successive samples for 44 women with symptomatic intrauterine pregnancies*

	Group 1	*Group 2*	*Group 4*	*Group 3*
	1.53 (5-48)	2.04 (3-2013)	1.63 (2-3250)	2.42 (6-571)
	2.42 (3-214)P	1.47 (2-1512)	1.89 (7-648)	1.68 (7-315)
	3.93 (2-168)B	2.51 (5-1364)	1.32 (5-1167)	1.65 (5-234)
	2.01 (3-222)	1.43 (2-1925)	1.71 (5-1104)	1.58 (5-309)
	1.66 (5-73)	1.92 (2-3172)	1.53 (3-1980)	2.82 (6-364)K
	2.04 (2-523)	2.56 (5-1166)	1.21 (3-1637)	1.54 (2-847)
	1.20 (2-255)		2.18 (6-2247)	1.41 (4-929)
	1.22 (2-336)		3.10 (4-3674)K	1.53 (7-389)
	1.31 (4-127)		1.27 (2-4053)	1.59 (7-429)
			1.41 (2-3020)	2.79 (6-548)K
			1.13 (3-2764)	1.62 (7-131)
			1.30 (5-892)	1.55 (6-491)
			2.03 (5-1335)	1.70 (5-472)
			1.46 (5-1647)	
			4.69 (3-4992)B	
			2.55 (4-4883)	
Mean	1.92	1.99	1.90	1.84
SD	0.86	0.49	0.92	0.49

*hCG doubling times for patients in groups 1, 2, and 4 of Pittaway et al. Figures in parentheses are the sampling intervals used in the slope estimates and the initial hCG values, respectively. Abnormal doubling times are indicated as P by the criteria of Pittaway et al., K by the criteria of Kadar and Romero (>2.7 days), and B by both criteria.

a clinical problem and in which serial hCG testing has proved useful. This is partly because the authors have selected asymptomatic patients for study, partly because they included both abortions and ectopic pregnancies under the term "abnormal pregnancy," and partly because they use serial hCG testing at hCG levels well above 6500 mIU/ml when a sonographic diagnosis of ectopic pregnancy can be reliably made or at least made more reliably than by serial hCG testing.[3,5] Some of our reservations about the methods used by Pittaway et al. to derive their multiple nomograms have been voiced elsewhere.[12] Nonetheless, if the log hCG-time relationship in early pregnancy is truly quadratic rather than linear, slope estimates based on paired samples will be influenced by both the initial hCG level and the time interval between the samples, and our earlier recommendations about how serial hCG testing should be carried out in practice might require revision. To address this question, we attempted in this article (1) to duplicate the findings of Pittaway et al. among symptomatic women with intrauterine pregnancies who did not abort, (2) to determine whether estimates of doubling time of hCG are influenced by the initial hCG and sampling interval, and (3) to compare the efficacy of the authors' multiple nomograms with our criteria for diagnosis of ectopic pregnancy among women with surgically proved ectopic pregnancies.

Material and methods

The data on the women who had intrauterine pregnancies were initially collected over an 11-month period in an effort to test our screening method subsequent to its formulation. Twenty patients, culled retrospectively from our hCG records, were subsequently added to the 24 patients who accrued over this period. The 24 prospectively followed patients were the subject of a separate report.[13] All patients were symptomatic when first seen, complaining of pain and/or abnormal vaginal bleeding, and each patient subsequently had an intrauterine pregnancy that progressed beyond the first trimester. The initial serum hCG value was below 6000 mIU/ml in all cases, and sonography was nondiagnostic. Two blood samples were obtained from 24 women, and three or more samples were obtained from 20 women. The final hCG value used in the determination of the doubling time was below 6500 mIU/ml in 25 patients, between 6500 and 9250 mIU/ml in 12 patients, and between 12000 and 18000 mIU/ml in seven patients. The hCG assay used at the Yale–New Haven Hospital has been previously described.[4] However, in the original description of our assay, the standard was erroneously stated as the second instead of the first internal reference preparation. (A second internal reference preparation does not, of course, exist, as discussed fully elsewhere.[14])

With the use of the first two available hCG values from each patient, the slope of the log hCG time curve was derived as: $\ln(hCG[2]/hCG[1]) \div$ sampling interval, and the doubling time calculated from $\ln 2/\text{slope}$ (where ln denotes log e). In four cases, the doubling time was calculated from the first and third hCG value since the first two samples had been drawn 1 day apart.

The data were then grouped according to criteria adopted by Pittaway et al.[10] as follows: group 1, both hCG values <1200 mIU/ml; group 2, both hCG values between 1200 and 6000 mIU/ml; group 4, initial hCG value between 600 and 6000 mIU/ml, with the final hCG value >6000 mIU/ml; group 3, initial hCG value <1200 mIU/ml, with the final hCG value > 1200 mIU/ml but <6000 mIU/ml. (One patient with a final hCG value of 6520 mIU/ml and one patient with an initial hCG value of 1166 mIU/ml were retained in group 2 to increase the size of that group.) The Kruskal-Wallis H statistic was calculated to test the null hypothesis that the three groups of doubling times (1, 2, and 4) were samples from the same population.

A quadratic ln hCG-time relationship predicts that the slope of the regression line is a linear function of the sampling interval and a transcendental (square root) function of the initial hCG, with no interaction occurring between the two terms. For example, the model of Pittaway et al. is exactly equivalent to b =

$\sqrt{(0.35 - 0.04H)} - 0.01S$, where b = slope, H = initial ln hCG, and S = sampling interval in days. Therefore the slope of the ln hCG-time curve for each patient was regressed on the sampling interval, and the initial hCG value was transformed in several ways. A model with initial hCG treated as a dummy variable was also fitted to the data. For this analysis, the data were dichotomized at 600 mIU/ml. Values below this figure were assigned a value of 1, and those above this figure were assigned a value of 2. The slope was also regressed separately on initial hCG and sampling interval; when the sampling interval was considered, a regression line was fitted to the entire data and to the <600 and >600 mIU/ml strata separately.

The additional analyses carried out on the data were as follows. First, for patients with more than two hCG values, successive estimates of the doubling time were made, and the difference between them was used to test the null hypothesis that this difference was zero. A two-tailed *t* test was used after the Kolmogorov-Smirnov test of the normality assumption required for the *t* test had been carried out. Second, for patients with identical sampling intervals, the doubling times were regressed on the initial hCG value (Spearman's rank-correlation coefficient). Third, to determine what proportion of total variability in the data was attributable to between-patient variation, the data were categorized by the sampling interval into four groups: 2 days, 3 and 4 days, 5 days, and 6 and 7 days, and one-way analysis of variance was performed assuming a random effects model. Finally, for each of three women who had five, seven, and nine hCG values determined, a linear and quadratic regression model was fitted separately to the data, and the extra sum of squares due to the quadratic term was tested for statistical significance by the F test. Standard TI 59 calculator programs were used to construct regression models. The significance of the regression sum of squares was tested after an analysis of variance table was constructed by the F test. Residuals were obtained for each regression model and plotted against the predicted value of the slope and against each independent variable. The plots were examined for any systematic trend that might indicate departure from linearity or homoscedasticity. Standardized residuals were also examined to check on the normality assumption implicit in the use of the F statistic. Estimates of pure error were obtained whenever replicate observations were available and used to test for lack of fit of the various regression models.

The patients with ectopic pregnancies accrued over a 3-year period (July 1980 to June 1983). Patients who had falling hCG values or who had sampling intervals of 1 day only or >7 days were excluded. The women were classified into the five groups of Pittaway et al., and the doubling time of each patient was compared with the authors' published limits for each group,[11] as well as our criteria, which represents a doubling time of 2.7 days.[3] Twenty-six patients were included in a previous report.[13]

Table II. The independent variables used in regression on doubling times of hCG*

Independent variable(s)	*Overall F test (df)*	*Lack-of-fit F test*	R^2
SI	1.72 (1,42)	1.92 (5,37)	0.04
SI (<600 mIU/ml)	1.22 (1,18)	1.81 (4,18)	0.0007
SI (>600 mIU/ml)	2.74 (1,22)	0.32 (4,18)	0.01
$\sqrt{H}$	0.22 (1,42)		0.005
H	0.22 (1,42)		0.005
SI, H	1.30 (2,41)		0.06
SI, $\sqrt{H}$	1.30 (2,41)		0.06
SI, Z	0.91 (2,41)	0.98 (9,32)	0.04

df = Degrees of freedom (numerator, denominator); SI = sampling interval; H = ln hCG; Z = 1 if hCG <600 mIU/ml; Z = 2 if hCG >600 mIU/ml.

*The independent variables in the regression models examined are shown together with the overall F statistic for the model, the lack-of-fit F statistic when replicate observations were available, and R, the squared correlation coefficient. The dependent variable in each model was b, the slope of the ln hCG time curve. 100R gives the percentage of the total variation in b attributable to variations in the independent variables, that is, the amount of variability explained by the model.

Results

The doubling time of hCG for each patient who had an intrauterine pregnancy, which was classified according to the groups of Pittaway et al., is shown in Table I, along with the initial hCG value and the sampling interval. There was no statistically significant difference between groups 1, 2, and 4 (H = 1.2). The doubling time for the entire study population was 1.9 days; five of the 44 patients (11.4%) had a subnormal increase in hCG values by our previously defined criteria.[3]

None of the several regression models examined fitted the data, as judged by the overall F test for the regression sum of squares at the 5% significance level. The F value for each model is given in Table II, along with the lack of fit F test where this could be derived, and the square of the correlation coefficient, R^2. (100 R^2 gives the proportion of the variability in the dependent variable explained by regression, that is, due to variation in the independent variable(s)). Both independent variables were entered into the model even though when examined separately neither explained a significant amount of the variability in the slope estimates.

Table III shows the slopes of the ln hCG-time curve obtained for four different sampling interval groups. Days 3 and 4 and 6 and 7 were combined to increase

Table III. The slope of the ln hCG-time curve for 44 women with symptomatic intrauterine pregnancies stratified by sampling interval*

	Sampling interval			
	2 days	*3 and 4 days*	*5 days*	*6 and 7 days*
	0.547	0.287	0.451	0.448
	0.360	0.339	0.471	0.245
	0.497	0.344	0.421	0.318
	0.425	0.453	0.437	0.413
	0.173	0.572	0.408	0.366
	0.597	0.615	0.404	0.452
	0.339	0.528	0.525	0.435
	0.579	0.491	0.271	0.427
	0.567	0.221	0.529	0.248
	0.451	0.271	0.276	0.287
	0.485	0.148	0.340	
			0.474	
Mean + SD	0.456 + 0.13	0.388 + 0.15	0.417 + 0.08	0.364 + 0.08

*The slope of the ln HCG time curve stratified by the sampling interval used in making the slope estimate. The variation between the four groups accounted for 2.28% of the total variability in slope.

the size of the groups. The differences between the four groups in the mean slope did not reach statistical significance irrespective of whether parametric ($F_{3,40}$ = 1.26) or nonparametric (H = 4.35, df = 3) analysis of variance was used; 2.28% of the variability in the data was attributable to differences in sampling interval; the remainder was between patient variation.

The difference between successive estimates of doubling time (second minus first) for each of 20 patients in whom >two hCG determinations had been performed was 0.67 (5/2), 0.88 (4/3), −0.14 (5/2), 0.38 (3/5), 0.24 (2/1), 0.64 (2/6), −0.29 (3/3), 1.35 (7/4),* 1.5 (5/8),* −0.28 (6/6),* 2.65 (1/1), 0.21 (3/4), 0.42 (1/4), 0.05 (2/3), 0.05 (2/4), 0.34 (1/4), −1.69 (2/2), 0.39 (5/3),* 0.62 (5/2), −0.30 (1/1) days, respectively. The figures in parentheses are the sampling intervals from which successive estimates of the doubling time were made, and the asterisks indicate the patients who had final hCG values above 20,000 mIU/ml. The mean difference ± SD was 0.38 ± 0.86. A two-tailed *t* test just failed to reject the null hypothesis that the true difference was zero (t = 1.98, $0.1 < p < 0.05$). The power of the test was 81% calculated for the expected mean difference predicted by Pittaway et al. Before the *t* test was performed, the Kolmogorov-Smirnov statistic was determined to check on the normality assumption required for the *t* test and could not be rejected even at the 20% significance level (D = 0.17). The hypothesis that the sample came from a normal population with zero mean could also not be rejected (D = 0.22), corroborating the result of the *t* test. The correlation between the doubling time and the initial hCG value could be examined for fixed sampling intervals of 2 and 5 days. The number of patients in each group was 11 and 12, respectively, and the corresponding r values were −0.05 and 0.2, respectively, neither of which is significantly different from zero.

The linear model, fitted to the five or more data points obtained from each of three patients, fitted the data very well in each case; the correlation coefficients were 0.996, 0.999, and 0.999, respectively. The extra sum of squares due to the quadratic term did not approach statistical significance in any of the three cases.

The doubling time of hCG for each of the 44 patients with ectopic pregnancies is shown in Table IV, along with the initial hCG value and the sampling interval. Four patients could not be classified into one of the groups for which the authors have published nomograms (group 3).[10] Of the 33 patients in groups 1, 2, and 4, 26 (79%) had abnormal doubling times of hCG as determined by the criteria of Pittaway et al., and 25 (76%) had abnormal values as determined by our criteria (76%): Of the 31 women with intrauterine pregnancies in the same groups (Table I), three (9.7%) had abnormal values as determined by both criteria. If group 5 is included, as was the case in the comparison carried out by Pittaway et al., then of the 40 patients with ectopic pregnancies, 31 (78%) had abnormal doubling times as determined by the criteria of Pittaway et al., whereas 32 (80%) had abnormal doubling times as determined by our criteria. Finally, if due consideration is given to those patients in group 3 for whom nomograms have not been published, then in the hCG range in which doubling time measurements of hCG are indicated in practice, the multiple nomograms identified 26 of 33 (79%) ectopic pregnancies with rising hCG titers as abnormal, whereas the single critical value of 2.7 days identified 28 of 37 (76%) patients as abnormal.

Table IV. hCG doubling times, initial hCG values, and sampling intervals between successive samples for 44 women with ectopic pregnancies*

Group 1	*Group 2*	*Group 3*	*Group 4*	*Group 5*
1.5 (4-60) B	3.9 (2-2520)	3.8 (4-787)	3.7 (4-5676)	12.0 (4-8448)
2.8 (4-104)	3.7 (2-3617)	1.8 (4-288)	2.1 (2-4352)B	2.8 (2-7102)P
2.8 (2-83)	11.7 (3-1197)	7.6 (5-936)	1.8 (3-2286)B	7.1 (2-13127)
1.7 (4-195)B	2.3 (2-1633)B	13.8 (7-1000)	1.7 (3-3744)B	10.4 (2-14000)
3.6 (4-143)	2.8 (2-3152)		2.1 (2-4352)B	8.2 (4-12400)
2.5 (4-71) K	6.1 (2-1299)			5.3 (2-11200)
20.9 (2-102)	4.0 (2-1200)			3.5 (3-6889)P
4.3 (2-603)	14.0 (2-1348)			
13.1 (3-145)	3.2 (2-3425)			
40.0 (4-981)	4.7 (3-1200)			
3.2 (5-908)	19.7 (7-1200)			
3.4 (7-83)	32.7 (6-1434)			
11.8 (2-160)	9.2 (5-1604)			
6.3 (6-600)				
3.7 (7-262)				

*HCG doubling times of patients with ectopic pregnancies classified into groups 1 to 5 of Pittaway et al. Normal values are indicated as P by the criteria of Pittaway et al., K by the criteria of Kadar and Romero, and B by both criteria.

Comment

In this study, we have been unable to duplicate the finding by Pittaway et al. that the doubling time of hCG varies significantly during early pregnancy. A number of possible reasons account for this discrepancy, but in our view the most likely explanation is that Pittaway et al. included several cases in their regression analysis with hCG values well in excess of the level above which the doubling time of hCG increases and that the log hCG-time relationship is no longer exponential.[6-8] The most plausible alternative explanation is that the discrepancy reflects inherent differences between women with intrauterine pregnancies who develop symptoms of pain and/or bleeding and those who do not. Inasmuch as the variance of the doubling times of hCG among our patients was fivefold to ninefold greater than that reported by Pittaway and Wentz,[11] some differences certainly exist. Nonetheless, the mean doubling time reported for group 3 by these authors was 1.8 days. Their group 3 was formed by amalgamating groups 1 and 2 and adding four patients who did not fall into either of these categories. The mean doubling time for groups 1, 2, and 3 combined in the present study was 1.9 days. Thus, regarding the mean doubling time of hCG, our respective samples were very similar. Perhaps further examination of this question may be warranted, but symptomatic women with intrauterine pregnancies and not women with normal pregnancies are clearly the ones requiring differentiation from those patients who have ectopic pregnancies; thus, in our opinion, they, not asymptomatic women, provide the appropriate control group from which the doubling time of hCG should be derived.

The suggestion that the use of multiple nomograms will improve the diagnosis of ectopic pregnancy also finds little support in this study. Certainly on theoretical grounds this would not be the case even if the true hCG-time relationship was quadratic and a linear approximation to it was used and vice versa. It would also be necessary to postulate that the overlap in the doubling times of hCG for intrauterine and ectopic pregnancies is different at different hCG values. This is because, irrespective of what the true log hCG-time relationship is, if the overlap is the same, one would simply be operating at a lower point of the receiver operating characteristics curve at low hCG values (resulting in fewer false positive and fewer true positive results) and at a higher point on the curve at higher hCG values (resulting in more false positive and more true positive results) by using a single rather than multiple nomograms.[9] In fact this is very well illustrated in this study. In group 1, our nomogram identified one less ectopic pregnancy but also one fewer false-positive result than the multiple nomograms. Conversely, in group 4, one more false-positive result was picked up, but by chance an additional ectopic pregnancy was not identified, which should cause no surprise given that this group had only five patients. However, inspection of group 5 should serve to convince those not familiar with the receiver operating characteristics curve principle that the true positive rate must increase in the long run at higher hCG values if our criteria are used. It can be seen that in this hCG range, five of the seven patients with ectopic pregnancies were identified by multiple nomograms as having abnormal results, whereas every patient had abnormal doubling times by our criteria.

The relationship between the log hCG and the time elapsed after the last menstrual period or basal body temperature shift could not be examined in this study.

However, such a relationship is not of direct relevance to the use of serial hCG testing in practice, since one of the major advantages of the method is precisely that the date of the last menstrual period (which is frequently unknown or unreliable) is irrelevant. Furthermore, the doubling time estimate of hCG derived from a regression line (which represents a cross-sectional analysis) will not be necessarily comparable with that obtained by averaging the hCG doubling time derived separately for each patient from serial values (a longitudinal analysis), at least not unless the sample size is very large. Thus the regression line per se is only of theoretic interest, and certainly the nomograms of Pittaway et al. were not derived from it. Nonetheless, a quadratic model predicts that slope or doubling time estimates of hCG will depend on the initial hCG and the sampling interval used and raises a question of practical interest that was investigated in several different ways.

When the two independent variables (initial hCG and sampling interval) were examined separately, it was found, in the hCG range studied, that the initial hCG had virtually no effect on the estimate of slope. This was true even when only cases with identical sampling intervals were considered. Similarly, the sampling interval used to assess the slope of the log hCG-time curve did not influence the estimate of slope significantly. This was again true irrespective of whether the entire data were considered or the analysis was confined to the two hCG strata, <600 and >600 mIU/ml. Therefore, not surprisingly, none of our quadratic equivalent models yielded a significant overall F test. Clearly, if the log hCG-time relationship in early pregnancy is quadratic, one would expect the slope estimates to be influenced by the sampling interval and the initial hCG. Failure to detect such a dependency, while it does not disprove, argues against a quadratic log hCG-time relationship. Furthermore, although only three patients could be studied, a linear model fitted the longitudinal data extremely well, and a quadratic term did not even approach statistical significance. In addition, successive estimates of doubling time for patients showed a far smaller tendency to increase than the results of Pittaway et al. would suggest, and since 12 of the final values were above the discriminatory zone, with four values above 20,000 mIU/ml, the small positive trend is not surprising.[6-8]

Therefore, in conclusion we have been unable to confirm that the diagnosis of ectopic pregnancy can be improved significantly by the use of multiple nomograms for the doubling time of hCG. In addition, the slope estimates obtained for our patients were uninfluenced by the initial hCG or the sampling interval used. Therefore, the suggestion that multiple nomograms be adopted in clinical practice appears to be premature and is likely to lead to difficulties, not only because they are much more complicated to use but also because many cases will not readily fit into the appropriate categories (17 of 88 in the present study). Although the number of women that cannot be properly categorized may be reduced by adoption of a uniform sampling interval as we originally proposed, this problem cannot be entirely eliminated.

REFERENCES

1. Cartwright PS, DiPietro DL. Ectopic pregnancy: changes in serum human chorionic gonadotropin concentration. Obstet Gynecol 1984;63:76.
2. Holman JF, Tyrey EL, Hammond CB. A contemporary approach to suspected ectopic pregnancy with use of quantitative and qualitative assays for the B-subunit of human chorionic gonadotropin and sonography. AM J OBSTET GYNECOL 1984;150:151.
3. Kadar N, Caldwell BV, Romero R. A method of screening for ectopic pregnancy and its indication. Obstet Gynecol 1981;58:162.
4. Kadar N, DeVore G, Romero R. Discriminatory hCG zone: its use in the sonographic evaluation for ectopic pregnancy. Obstet Gynecol 1981;58:156.
5. Romero R, Kadar N, Jeanty P, et al. Diagnosis of ectopic pregnancy: value of the discriminatory hCG zone. Obstet Gynecol 1985;66:357.
6. Marshall JR, Hammond CB, Ross GT, Jacobson A, Rayford P, Odell WD. Plasma and urinary chorionic gonadotropin during early human pregnancy. Obstet Gynecol 1968;32:760.
7. Kosasa TS, Taymor ML, Goldstein DP, et al. Use of a radioimmunoassay specific for human chorionic gonadotropin in the diagnosis of early ectopic pregnancy. Obstet Gynecol 1973;42:868.
8. Goldstein DP, Aono T, Taymor ML, et al. Radioimmunoassay for chorionic gonadotropin activity in normal pregnancy. AM J OBSTET GYNECOL 1968;102:110.
9. Kadar N, DeCherney AH, Romero R. Receiver operating characteristics (ROC) curve analysis of the relative efficacy of single and serial chorionic gonadotropin determinations in the early diagnosis of ectopic pregnancy. Fertil Steril 1982;37:542.
10. Pittaway DE, Reish RL, Wentz AD. Doubling times of human chorionic gonadotropin increase in early viable intrauterine pregnancies. AM J OBSTET GYNECOL 1985; 152:299.
11. Pittaway DE, Wentz AC. Evaluation of early pregnancy by serial chorionic gonadotropin determinations: a comparison of methods by receiver operating characteristic curve analysis. Fertil Steril 1984;43:529.
12. Kadar N. The log hCG-time relationship in early pregnancy [Letter to the Editor]. AM J OBSTET GYNECOL 1986;154:692.
13. Romero R, Kadar N, Copel J, et al. Serial hCG testing as a diagnostic tool in ectopic pregnancy. AM J OBSTET GYNECOL 1986;153:73.
14. Romero R, Kadar N. Letter to the Editor. Obstet Gynecol 1986;68:441.

Pregnancy among obstetricians: A comparison of births before, during, and after residency

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Questionnaires were sent to 1025 female board-certified obstetricians, and information was retrieved about pregnancy outcome. A total of 454 pregnancies, one third of which occurred during residency, were evaluated, and the relationship between pregnancy outcome and residency was assessed. Children of primiparous women who were delivered during or after residency had significantly lower mean birth weights than those who were delivered before residency ($p < 0.001$ and $p < 0.005$, respectively), whereas birth weights of infants born to multiparous women were not significantly different. The low birth weight rate (<2500 gm) was significantly increased during residency ($p < 0.002$), and infants born during residency were 7.5 times more likely to be growth retarded than those born outside residency ($p < 0.002$). The incidence of other pregnancy complications was not found to be increased during residency. Our data suggest a potentially negative impact of residency on the birth weights of infants born to female obstetricians in training. (AM J OBSTET GYNECOL 1987;157:79-83.)

Key words: Pregnancy complications, residency, intrauterine growth retardation

The number of women in residency programs has dramatically increased in the past decade. In some specialties such as pediatrics and obstetrics and gynecology, women now constitute between one third and one half of all residents.[1] Since residency occurs at the height of most women's reproductive cycle, an increasing number of pregnancies among residents can be anticipated. Accordingly, the problems of pregnancy during residency have received increased attention. Initial reports have centered on issues such as the perception of pregnant residents by others involved in residency and suggestions for improvements in working conditions during residency.[2] What has not been addressed, however, is the potential effects of residency on pregnancy outcome. Residency entails long periods of walking, running, and standing, frequent periods of sleep deprivation, and extremely long work weeks and thus is one of the most physically and emotionally demanding types of work. A house officer may spend between 80 and 120 hours per week in the hospital. Some epidemiologic studies have suggested that work during pregnancy may be associated with decreased newborn weight,[3, 4] and residency clearly involves an extreme amount of work. Furthermore, the house officer is frequently exposed to potential teratogens in the hospital such as anesthetic gases, cytotoxic drugs, infectious agents (e.g., cytomegalovirus and hepatitis), and radiation. Several reports have linked occupational exposure to cytotoxic agents[5] and anesthetic gases,[6] for example, to fetal loss.

Not only do residents work harder and longer than persons in most other jobs, but board requirements and peer pressure within training programs force many pregnant residents to continue their full work and night-call schedule until shortly before delivery. Whether this is compatible with optimal pregnancy outcome is not known.

Although there are several epidemiologic reports on the relationship between other types of work and pregnancy, the impact of residency on pregnancy outcomes has not been studied. The present study was designed to assess the impact of residency on pregnancy outcome.

Subjects and methods

Between August and November 1984, questionnaires were sent to the first 1025 female board-certified obstetricians published in the directory of the American Board of Obstetrics and Gynecology. Obstetricians were selected because it was felt that this group would be the most able to provide accurate information on pregnancy outcome and because they took part in one of the most arduous residencies. The questionnaires retrieved general medical and perinatal information such as date of birth, years of residency, maternal age at each pregnancy, medical and obstetric complications, infant birth weight, gestational age at birth, Apgar scores, mode of delivery, diet, pregnancy symptoms, exposure to hazards, history of smoking or alcohol, and

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Table I. Relationship between residency and pregnancies

	No. of deliveries	No. of spontaneous/induced abortions	Total n	Total (%)
Before residency	82	1	83	(18.3)
During residency	147	4	151	(33.3)
After residency	192	28	220	(48.4)
	421	33	454	(100%)

Table II. Relationship between residency and hours worked per week during pregnancy*

	Mean hr ± SEM	Range
Before residency	36.4 ± 4.7	0-100
During residency	78.9 ± 4.0	40-120
After residency	46.5 ± 4.6	0-100

*$p < 0.01$.

maternal weight gain. Women who had never been pregnant and those who were pregnant at the time they responded were excluded. Low birth weight was defined as any birth weight under 2500 gm. Preterm delivery was defined as any delivery before completion of the thirty-seventh week of gestation. Intrauterine growth retardation was defined as a birth weight that was under the tenth percentile for a given gestational age. Women were grouped into primiparous and multiparous, since average birth weights of subsequently born infants are known to be higher than those of firstborn infants.[2]

The chi-square test, Student *t* test, and Fisher's exact test were used for statistical evaluations.

Results

Four hundred ninety-nine questionnaires were returned for a return rate of 48.7%. The study population was made up of 218 women with 454 pregnancies. The other 281 patients had not delivered any children or were pregnant for the first time during the study.

Residency, pregnancy, and hours worked. Table I shows the timing of pregnancies in relation to residency. Of the 454 pregnancies reported, 18.3% occurred before residency, 33.3% occurred during residency, and 48.4% occurred after residency. There were 421 deliveries and 33 spontaneous or induced abortions. More women aborted after residency than before or during residency ($p < 0.01$). Table II shows the relationship between residency and hours worked during pregnancy. Residents worked more than twice as much as women who delivered before residency.

Birth weights. Table III shows the average birth weights of infants in relationship to residency. Primiparous women delivering during residency had the lowest overall birth weight. The average birth weight of firstborn infants delivered during residency was significantly smaller than the birth weight of firstborn infants delivered before residency (3146 ± 696 gm versus 3525 ± 455 gm, respectively; $p < 0.001$). Even though mean birth weights increased after residency, they were still lower than those of infants born before residency (3263 ± 556 gm; $p < 0.005$). Multiparous women delivering during residencies also had children with the lowest birth weights when compared with multiparous women delivering before or after residency, but these numbers did not reach statistical significance.

Low birth weight infants, preterm deliveries, and intrauterine growth retardation. Table IV shows the relationship between timing of pregnancy and the frequency of low birth weight, preterm births, and intrauterine growth retardation. The low birth weight rate for the study population was 5.9% (25/421). It was significantly higher for those subjects delivered during residency than for those delivered before or after residency (11.6% low birth weight during, 3.7% low birth weight before, and 2.6% low birth weight after residency; $p < 0.002$).

Because low birth weight infants include those infants delivered preterm and those with intrauterine growth retardation, we further evaluated these two categories. There was no significant differences in the rate of preterm deliveries in relation to residency (4.9%, 3.6%, and 4.2%; $p > 0.05$). However, infants delivered during residency were significantly more likely to be born with intrauterine growth retardation than those delivered before or after residency (8.2% intrauterine growth retardation during residency versus 1.2% intrauterine growth retardation before and 1.0% intrauterine growth retardation after residency; $p < 0.001$). Matched-pair analysis showed that other factors potentially affecting birth weight, such as prepregnancy weight, weight gain during pregnancy, race, and substance abuse, were not observed more frequently in patients with intrauterine growth retardation than in those without. We also attempted to correlate birth weights with hours worked per week and weeks off before delivery, but too few subjects reported working a "reasonable" amount of hours (under 80 hours/wk) during pregnancy or taking off more than 1 week before delivery to allow for comparison.

No other associations were noted. Specifically, the incidence of malformations, medical complications (e.g., hypertension and diabetes), obstetric complications (e.g., placenta previa and abruptio placentae), and pregnancy symptoms were not found more frequently during residency. Sixty-seven percent of the respondents noted that during pregnancy, they did not follow nutritional or rest guidelines that they recommend to others.

Table III. Relationship between residency and average birth weight

	Firstborn infants		*Subsequent births*	
	n	*Mean gm ± SD*	*n*	*Mean gm ± SD*
Before residency	46	3525 ± 455	36	3502 ± 586
During residency	101	3146 ± 696*	46	3334 ± 508
After residency	71	3263 ± 556†	121	3387 ± 461

*$p < 0.001$ for difference between before and during residency.

†$p < 0.005$ for difference between before and after residency.

Table IV. Relationship between residency, low birth weight infants, preterm deliveries, and intrauterine growth retardation

		LBW		*Preterm*		*IUGR*	
	Total n	*%*	*(n)*	*%*	*(n)*	*%*	*(n)*
Before residency	82	3.7	(3)	4.9	(4)	1.2	(1)
During residency	147	11.6	(17)	3.6	(7)	8.2	(12)
After residency	192	2.6	(5)	4.2	(8)	1.0	(2)
		$p < 0.002$		NS		$p < 0.001$	

LBW = low birth weight; IUGR = intrauterine growth retardation.

Table V shows the characteristics of all low birth weight deliveries.

Comment

Pregnant house officers face a dilemma when trying to decide when to stop working. The Council of Scientific Affairs of the American Medical Association has recommended that women with strenuous jobs (intermittent standing over 30 min/hr and repetitive stair climbing >4 times/8 hr), should not work beyond the twenty-eighth week of gestation.[7] Many obstetricians suggest that their patients stop working during the latter part of pregnancy and avoid becoming overtired. However, despite the fact that residency is clearly arduous labor, specialty boards frequently prohibit a resident from taking a leave for more than 6 weeks in any given year of training. As a consequence, a pregnancy leave beyond 6 weeks, even for reasons of disability, represents the potential loss of accreditation for the resident's year of training. Therefore, many female house officers choose to work until close to their expected date of delivery to save most of their leave time for after the delivery.

The results of our study suggest that residency training with its 80- to 120-hour weeks may be associated with adverse pregnancy outcome among female house officers. Average birth weights of infants born to primiparous women during residency were reduced by 379 gm compared with infants born before residency ($p < 0.001$) and by 117 gm compared with those delivering after residency. The low birth weight rate was significantly increased during residency (11.6% versus 2.9%; $p < 0.001$). Even though mean birth weights increased after residency, they were still significantly lower than before residency. This may suggest that the work load of female obstetricians even after residency continues to be substantial and impacts on neonatal birth weights.

It might be expected that physicians, who generally belong to upper socioeconomic groups, would have more favorable perinatal outcomes than women belonging to socioeconomically disadvantaged groups. However, the low birth weight rate of 11.6% among infants born to female house officers delivering during residency was similar to that of women of lower socioeconomic groups and twice as high than the 5.9% low birth weight rate reported among women of similar socioeconomic status on a national level.[8] When compared with normal birth weight infants, those with low weight at birth are almost 40 times more likely to die within the neonatal period[9] and three times as likely to have adverse neurologic sequelae.[10]

Several studies have attempted to assess the impact of work on pregnancy. Saurel and Kaminski[11] reported a slightly lower rate of intrauterine growth retardation among employed women, and they suggested that evidence from their research was more in favor of a positive rather than an adverse effect of work on pregnancy outcome. However, the authors noted that employed women in their study had better antenatal care than nonemployed women and were given 6 weeks of antenatal and 8 weeks of postnatal leave. Furthermore, working women may differ from nonworking women in many respects other than their occupation. For ex-

Table V. Characteristics of low birth weight infants

Birth	*Birth weight (gm)*	*Gestational age (wk)*	*5-min Apgar score*	*Maternal age (yr)*	*Delivery mode*	*Birth order*
Before residency	2041	34	8	20	Vaginal	1
	1500	30	9	30	Vaginal	2
	2325	36	9	22	Vaginal	2
During residency	2098	36	9	33	Cesarean	1
	2013	32	9	30	Cesarean	1
	2381	39	9	28	Vaginal	1
	1786	30	9	29	Cesarean	1
	1950	34	9	28	Cesarean	1
	680	24	0	31	Vaginal	1
	964	38	0	31	Cesarean	1
	2268	38	10	32	Vaginal	1
	2415	38	9	31	Vaginal	1
	2184	38	8	26	Vaginal	1
	2433	35	10	31	Vaginal	1
	1984	37	9	31	Cesarean	1
	789	36	0	27	Vaginal	1
	1673	39	9	30	Vaginal	2
	2268	40	10	34	Vaginal	2
	2098	38	10	30	Vaginal	2
	2325	38	10	29	Vaginal	2
After residency	1000	28	3	38	Vaginal	1
	1219	32	0	38	Vaginal	1
	2495	40	9	37	Cesarean	1
	1490	33	5	33	Cesarean	2
	2211	43	8	33	Vaginal	3

ample, dietary intake, socioeconomic status, race, and family size may differ between the two groups. Working women may have better access to adequate perinatal care than nonworking women. There is also the "healthy worker effect" that is, women in the work force may represent an inherently more fit subpopulation of pregnant women.

In contrast, findings from other studies suggest that work may be associated with decreased fetal birth weights. Alegre et al.[3] found that work during pregnancy was associated with a 200 gm decrease in newborn weight, and other studies confirmed that hard work may decrease fetal growth and increase fetal and neonatal mortality.[12] Naeye and Peters[4] reported a reduction in birth weight of between 150 and 400 gm among employed women. Growth retardation was most severe when women had standup jobs, continued work until near term, were hypertensive, or had children at home. Obstetric residents would have to be considered in the "hard-working" category. Female residents from other disciplines may also be prone to the same risks, but obstetricians were studied, since it was felt that they were best able to give a reliable obstetric history.

There are several explanations for decreased fetal growth when a mother routinely performs strenuous activity. Physical work involves muscle action that causes sympathetic vasomotor activity in the muscle tissue. This in turn might increase blood flow to the working muscle and absolutely or relatively diminish blood flow to the placenta. Maternal exercise, for example, is known to reduce uteroplacental blood flow,[13] and maternal plasma volume and cardiac output are lower in the upright than in the recumbent position.[14] The hard work performed by residents frequently in the upright position might potentiate intrauterine fetal growth retardation.

A potential bias in this study may have been a higher rate of response to the questionnaire among women having adverse pregnancy outcomes. Although this bias may have had some effect, intrauterine growth retardation was the only adverse outcome reported with increased frequency during residency. There was an equal distribution of other adverse outcomes such as preeclampsia, preterm labor, and diabetes. In addition, the rates of adverse pregnancy outcome for our study population are not excessive and compare well with those reported in the literature for a population of similar socioeconomic status.

In conclusion, our study suggests that a female house officer is at increased risk of experiencing adverse pregnancy outcome. They were more likely to deliver a low birth weight, growth-retarded infant during residency than before or after residency. Because there is an association between low birth weight infants and fetal and neonatal morbidity and mortality, the results of this study have implications for both women considering pregnancy during obstetric residency and residency program directors who establish work and leave policies for parturients in their programs. Work and leave policies in individual residency programs, however, cannot supersede the leave policy established by specialty boards. The policy of the American Board of Obstetrics and Gynecologists does not allow leave time beyond 6 weeks for any given year of training.

Some suggestions have recently been made about ways to better accommodate a pregnant resident.[2] Modifications of work schedules during pregnancy with more time off may be beneficial to the pregnant physician.[15] Alternatively, light duty (electives and pathology) without being on call might be assigned in the latter half of pregnancy.

This study also has implications for obstetricians who care for pregnant residents. It is their responsibility to make suggestions to their patients regarding stopping or modifying work. Although a leave of 6 to 8 weeks is generally suggested for normal, uncomplicated pregnancies,[16] in cases involving heavy work such as residency, suggested antepartum leave time may need to be extended to prevent adverse pregnancy outcome.

Since increasing numbers of women have entered training programs, further exploration of the questions of what to do when pregnancy occurs during residency and how to protect the fetus needs to be undertaken.

REFERENCES

1. Crowley AE. Summary statistics on graduate medical education in the United States. JAMA 1984;252:1545.
2. Sayres M, Wyshak G, Denterlein G, et al. Pregnancy during residency. N Engl J Med 1986;314:418-23.
3. Alegre A, Rodriguez-Escudero FJ, Cruz E, et al. Influence of work during pregnancy on fetal weight. J Reprod Med 1984;29:334-6.
4. Naeye RL, Peters EC. Working during pregnancy; effects on the fetus. Pediatrics 1982;69:724.
5. Selevan SG, Lindbohm M-L, Hornung, R, et al. A study of occupational exposure to antineoplastic drugs and fetal loss in nurses. N Engl J Med 1985;313:1173-8.
6. Cohen EN, Bellville JW, Brown BW. Anesthesia, pregnancy, and miscarriage: a study of operating room nurses and anesthetists. Anesthesiology 1971;35:343-7.
7. Effects of pregnancy on work performance. Council on scientific affairs. JAMA 1984;251:1995-7.
8. Health, United States, 1982. Washington, DC: Government Printing Office, 1982; Department of Health and Human Services publication no. (PHS)83-1232.
9. Shapiro S, McCormick MC, Starfield BH, et al. Relevance of correlates of infant deaths for significant morbidity at 1 year of age. AM J OBSTET GYNECOL 1980;136:363.
10. Papile L-A, Munsick-Bruno G, Schaefer A. Relationship of cerebral intraventricular hemorrhage and early childhood neurologic handicaps. J Pediatr 1983;103:273.
11. Saurel MJ, Kaminski M. Pregnant women at work. [Letter.] Lancet 1983;1:475.
12. Paul AA, Muller EM, Whitehead RG. The quantitative effects of maternal dietary energy intake on pregnancy and lactation in rural Gambian women. Trans R Soc Trop Med Hyg 1979;73:686.
13. Pomerance J, Gluck L, Lynch VA. Maternal exercise as a screening test for uteroplacental insufficiency. Obstet Gynecol 1974;44:383.
14. Suonio S, Simpanen AL, Olkkonen, et al. Effects of the left lateral recumbent position compared with the supine and upright positions on placental blood flow in normal late pregnancy. Ann Clin Res 1976;8:22.
15. Schwartz RW. Pregnancy in physicians: characteristics and complications. Obstet Gynecol 1985;66:672.
16. American College of Obstetrics and Gynecology: Pregnancy, work, and disability. Washington DC: American College of Obstetricians and Gynecologists, 1980.

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Cholesteryl palmitate as a predictor of fetal lung maturity

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Cholesteryl palmitate was measured by thin-layer chromatography in 98 amniotic fluid samples obtained from pregnancies ranging from 15 to 40 weeks. Pregnancies complicated by diabetes mellitus or Rh sensitization were excluded. Cholesteryl palmitate concentration increased with gestational age from values <5 μg/ml at 15 weeks to maximum values of 384 μg/ml at term. Seventy-three patients were delivered of their infants within 24 hours of sampling. The mean concentration of cholesteryl palmitate for 55 infants without respiratory distress syndrome was 123.70 ± 12.21 μg/ml (SD). For neonates that developed respiratory distress syndrome ($n = 18$), the mean concentration was 22.66 ± 6.16 μg/ml (SD). This difference is significant ($p < 0.001$). For all patients delivered, respiratory distress syndrome was not seen when the cholesteryl palmitate concentration was >41 μg/ml (mean + 3 SD). All infants with a concentration below 38 μg/ml developed respiratory distress syndrome. We conclude that measurement of cholesteryl palmitate concentration in amniotic fluid might be another reliable method of assessing fetal lung maturity and may often improve specificity when compared with other tests of fetal lung maturity. (AM J OBSTET GYNECOL 1987;157:84-8.)

Key words: Cholesteryl palmitate, amniotic fluid, fetal lung maturity, respiratory distress syndrome

Respiratory distress syndrome (RDS) remains an important cause of neonatal morbidity and mortality. Currently available methods for assessing fetal lung maturity including quantitation of the phospholipid content of amniotic fluid and determination of the physical properties of amniotic fluid accurately predict the absence of subsequent RDS but lack specificity. Thus investigators continue to search for a test that is a better predictor of RDS. In our laboratories, Alvarez et al.,[1] studying amniotic fluid samples by thin-layer chromatography, have observed large amounts of nonpolar or neutral lipids. Cholesteryl palmitate was identified as a major component of these lipids. Almost 40% of the total nonpolar lipid fraction of amniotic fluid at term corresponded to cholesteryl palmitate. Since most amniotic fluid lecithin at term is composed of saturated phosphatidylcholine esterified with palmitic acid[2] and because cultured human lung fibroblasts can transport and esterify cholesterol,[3] we hypothesized that a relationship could be demonstrated between fetal pulmonary maturation and the concentration of cholesteryl palmitate in amniotic fluid. We questioned whether the amniotic fluid concentration of cholesteryl palmitate could be used as a sensitive and specific predictor of fetal lung maturity. This article describes the analytic methods used and the results of our initial evaluation of the clinical correlation of cholesteryl palmitate and fetal pulmonary maturity.

Material and methods

Ninety-eight amniotic fluid samples were obtained at gestational ages from 15 weeks to term. Fluid was obtained by transabdominal amniocentesis performed for clinical indications. The population studied included both normal and high-risk pregnancies. Samples from pregnancies complicated by diabetes mellitus or Rh sensitization were excluded, as were samples contaminated by blood or meconium. Aliquots of amniotic fluid were sent immediately to the clinical laboratory for determination of the lung profile by a modification of the method described by Kulovich et al.[4] as well as a determination of the optical density at 650 nm.[5] A 1 ml aliquot was reserved for measurement of cholesteryl palmitate. Thin-layer plates (Whatman LK5) were obtained from Whatman, Inc., Clifton, New Jersey. Cholesteryl palmitate was purchased from Supelco, Inc., Bellefonte, Pennsylvania. All solvents were of the EM Omnisolv grade. All chemicals were reagent grade.

A Kontes Model 800 fiberoptic densitometer (Kontes, Vineland, New Jersey) interfaced with an HP3385A integrator was used for quantitation.

For the assay of cholesteryl palmitate, 20 to 40 μl of

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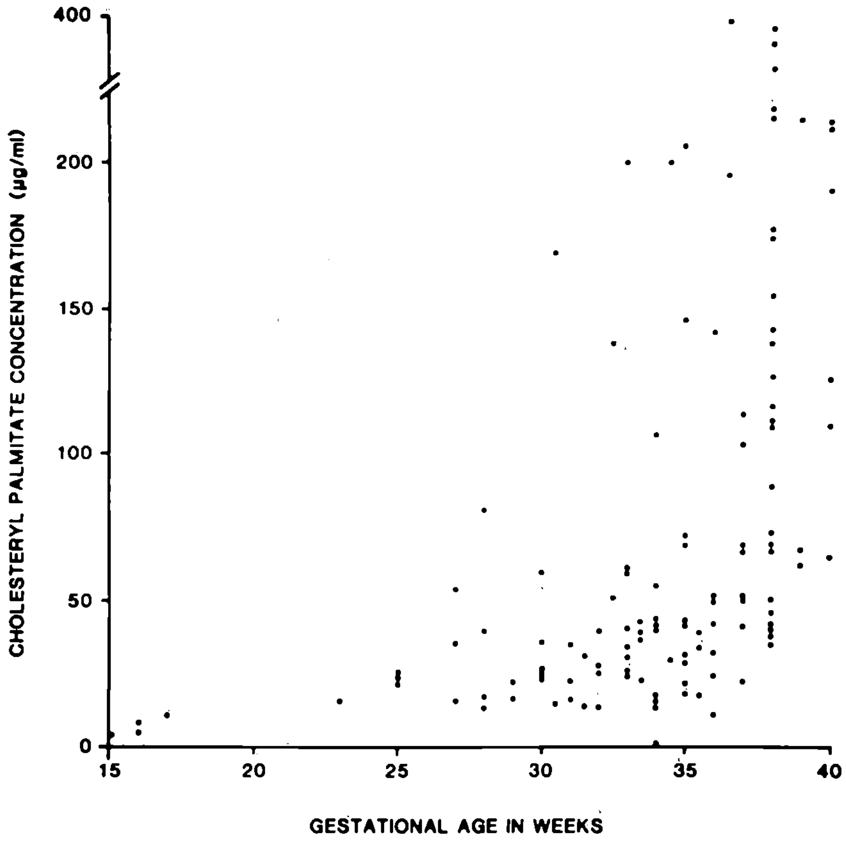

Fig. 1. Cholesteryl palmitate concentration as a function of gestational age.

amniotic fluid was applied directly to the preadsorbent portion of Whatman LK5 layers after the method described by Touchstone et al.[6] After drying under a hair dryer for 5 minutes, the chromatograms were predeveloped twice with chloroform-methanol (1 : 1) to the interface with the adsorbent, with drying after each. The chromatogram was then developed until the mobile phase (hexane-ether, 96:4) reached 1 cm from the top of the plate. Cholesteryl palmitate was applied to an outer lane as a reference. The chromatogram was then dried under a hair dryer and finally sprayed until wet with 10% copper sulfate in 8% phosphoric acid.[6] The chromatogram was allowed to dry for 5 minutes at ambient conditions and then heated in an oven at 170° C for 10 minutes. Quantitation was performed by scanning in the visible mode on the densitometer. The integrated areas were interpolated on a standard curve prepared from serial amounts of cholesteryl palmitate applied to the layer and carried through the procedure.

Results of the cholesteryl palmitate assay were not used in clinical management nor where the results reported to clinicians managing the patients. The presence of RDS was determined by a staff neonatologist who was not aware of the cholesteryl palmitate concentration. The diagnosis of RDS required signs of grunting, tachypnea, retraction, nasal flaring, and a reticulogranular ground-glass appearance with an air bronchogram on chest x-ray film, as well as the requirement of supplemental oxygen.

Results

Fig. 1 shows that the cholesteryl palmitate concentration increased with gestational age from values of <5 µg/ml at 15 weeks to maximum values of 384 µg/ml at term. For patients <32 weeks' gestation (n = 27) the mean cholesteryl palmitate was 29.35 ± 6.18 µg/ml (SEM). Samples from patients between 32 and 35 weeks (n = 27) contained a mean cholesteryl palmitate concentration of 56.17 ± 9.45 µg/ml (SEM). Fluid obtained from pregnancies >35 weeks (n = 43) had a mean cholesteryl palmitate concentration of 127.8 ± 1.51 µg/ml (SEM). Of 73 patients who were delivered within 24 hours after the measurement of amniotic fluid cholesteryl palmitate, 55 had infants without RDS (Table I). The mean concentration of cholesteryl palmitate for this group was 123.70 ± 12.21 µg/ml (SD). However, for neonates that developed RDS (n = 18), the mean cholesteryl palmitate concentration was 22.66 ± 6.16 µg/ml (SD). The difference in amniotic fluid cholesteryl palmitate concentration for in-

Table I. Cholesteryl palmitate concentration for 73 patients delivered within 24 hours of sample

	No RDS (*n = 55*)	*RDS* (*n = 18*)	*p Value**
Cholesteryl palmitate (μg/ml) (mean ± SD)	123.70 ± 12.21	22.66 ± 6.16	<0.001

*Student's *t* test.

Table II. Lecithin/sphingomyelin ratio, phosphatidylglycerol, optical density at 650 nm, and cholesteryl palmitate (mean ± 3 SD) for patients delivered

	Lecithin/sphingomyelin ratio		*Phosphatidylglycerol*		*Optical density at 650 nm*		*Cholesteryl palmitate*	
	≥2	<2	+	−	*≥0.15*	*<0.15*	*>41 μg/ml*	*≤41 μg/ml*
RDS (n = 18)	4	14	0	18	2	16	0	18
No RDS (n = 55)	53	2	41	14	41	14	51	4

fants with and without RDS was statistically significant (p < 0.001).

We then evaluated the risk for RDS in our study population, using the mean cholesteryl palmitate concentration at which RDS occurred, 22.66 μg/ml, +3 SD above the mean. At this value, ≤41 μg/ml but above 38 μg/ml, there were four infants. One had a cholesteryl palmitate concentration equal to 41 μg/ml and the other three had values of 40, 39.6, and 38.2 μg/ml (Fig. 2). Each infant (18/18), however, with an amniotic fluid cholesteryl palmitate concentration below 38 μg/ml subsequently developed RDS.

We compared the lung profile, the optical density at 650 nm, and a cholesteryl palmitate concentration of >41 μg/dl for all patients delivered within 24 hours of the amniocentesis. For neonates without RDS, 51 of 55 (93%) had a cholesteryl palmitate concentration >41 μg/ml. Only 41 of these 55 (75%) demonstrated phosphatidylglycerol (Table II). RDS resulted in 14/16 (88%) cases with an immature lecithin/sphingomyelin ratio, 18/32 (56%) without phosphatidylglycerol, 16/30 (53%) with an immature optical density at 650 nm, and 18/22 (82%) with a cholesteryl palmitate concentration below 41 μg/ml.

Comment

The lecithin/sphingomyelin ratio as described originally by Gluck et al.[7] remains the gold standard for the prediction of fetal lung maturity. However, only 30% to 40% of neonates with an immature lecithin/sphingomyelin ratio develop RDS.[8, 9] While the test is extremely good for predicting pulmonary maturity and allowing elective delivery at term, modern obstetric care and intervention for premature babies require a better predictor of RDS when the lecithin/sphingomyelin ratio is immature.

The lung profile as described by Kulovich et al.[4] has improved the specificity of the assay, but it is time-consuming, requires a larger sample of amniotic fluid, and must be performed by trained technicians. A number of other tests have been developed for ease and rapid availability of results; as is the case with the lecithin/sphingomyelin ratio, they are reliable with maturity but lack specificity.[10] In view of the above, Garite et al.[11] recently tried to reduce the number of falsely immature values by using a maturity cascade scheme combining three different tests: the shake test, fluorescence polarimetry, and lecithin/sphingomyelin ratio. However, only 59% of babies with an immature cascade developed RDS.

The concentration of cholesteryl palmitate increases with advancing gestational age. Maximum values are reached at term and the concentration curve of amniotic fluid cholesteryl palmitate is similar to that for lecithin.[6] The source of cholesterol esters in amniotic fluid is unknown. A recent study by Slotte et al.[3] has shown that the human lung is able to transport and esterify cholesterol. It is possible that this lipid could be a transport mode for palmitic acid and thus facilitate esterification to give saturated phosphatidylcholine, an important step in the synthesis of surfactant: An alternative mechanism could be that cholesteryl palmitate is involved in the storage of fatty acids in the lung for later transesterification to occur. The fetal lung can support active de novo fatty acid synthesis. This activity presumably increases toward term and decreases after birth.[12] Further investigation is required to elucidate the source and role of cholesteryl palmitate in surfactant synthesis.

The cholesteryl palmitate assay is simple, requiring only 20 to 40 μl of amniotic fluid. Acetone precipitation and centrifugation are not needed. Touchstone et al.[6]

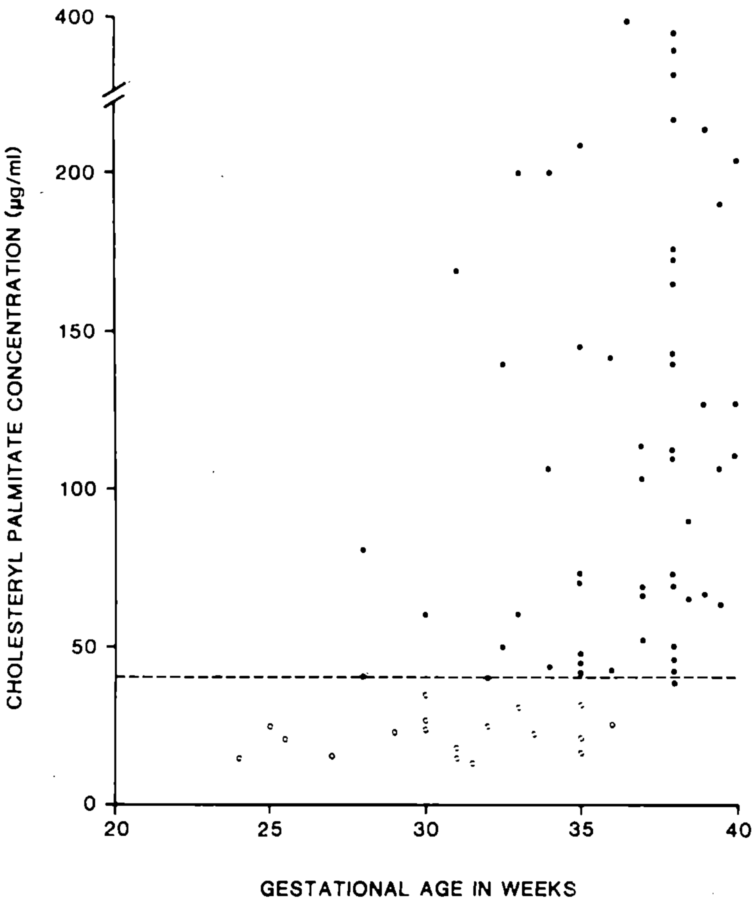

Fig. 2. Cholesteryl palmitate concentration for patients delivered within 24 hours of sample. ●, No RDS. ○, RDS.

have shown better results omitting acetone precipitation and using cupric sulfate for the detection of lipids separated on thin-layer chromatography. The assay takes 30 to 40 minutes, with the final values of cholesteryl palmitate obtained by densitometry. Cholesterol esters are present in blood attached to the lipoproteins.[13] Thus amniotic fluid contaminated by blood or meconium exhibited a greater concentration of cholesteryl palmitate. On the basis of this observation we feel that there is no advantage in measuring cholesteryl palmitate in the above situations.

In our retrospective study, we found that in normal pregnancies RDS was not observed with a cholesteryl palmitate concentration >41 μg/ml. Furthermore, all infants with a cholesteryl palmitate level below 38 μg/ml developed RDS. In terms of lung maturity cholesteryl palmitate measurement is as sensitive as the lecithin/sphingomyelin ratio in predicting the absence of RDS but demonstrated greater specificity with presence of RDS when the cholesteryl palmitate concentration was below 38 μg/ml. With a greater number of cases it may be that the sensitivity of the test will be the same as that of the lecithin/sphingomyelin ratio, but the specificity will be greatly increased.

Further studies are in progress to determine the role of cholesteryl palmitate in surfactant synthesis as well as its predictive value in high-risk pregnancies such as those complicated by diabetes mellitus.

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REFERENCES

1. Alvarez JG, Ludmir J, Lopez IM, Touchstone JC. Isolation of cholesteryl palmitate from amniotic fluid [Abstract 205]. In: Proceedings of the thirty-third annual meeting of the Society for Gynecologic Investigation, Toronto, Ontario, Canada, March 19-22, 1986.
2. Vollker DR, Lee TC, Snyder F. Fatty acid biosynthesis and dietary regulation in pulmonary adenomas. Arch Biochem Biophys 1976;176:753.
3. Slotte JP, Lundberg B, Bjorkerud S. Intracellular transport and esterification of exchangeable cholesterol in cultured human lung fibroblasts. Biochim Biophys Acta 1984;793:423.
4. Kulovich MV, Hallman MB, Gluck L. The lung profile. I. Normal pregnancy. AM J OBSTET GYNECOL 1979; 135:57.

5. Sbarra AJ, Michlevitz H, Selvaraj RJ, et al. Correlation between amniotic fluid optical density and L/S ratio. Obstet Gynecol 1976;48:613.
6. Touchstone JC, Levin SS, Dobbins MF, Matthews L, Beere PC, Gabbe SG. (3-sm-phosphatidyl) cholines (lecithins) in amniotic fluid. Clin Chem 1983;29:1951.
7. Gluck L, Kulovich MV, Borer RC, Brenner PH, Anderson GG, Spellacy WN. Diagnosis of the respiratory distress syndrome by amniocentesis. AM J OBSTET GYNECOL 1971; 109:440.
8. Haney D, Parkinson CE, Campbell S. Risk of respiratory distress syndrome. Lancet 1975;1:42.
9. Herbert WNP, Tyson J, Jimenez JM. Severity of respiratory distress syndrome with low lecithin : sphingomyelin ratio. Obstet Gynecol 1981;57:426.
10. Gabbe SG. Recent advances in the assessment of fetal lung maturity. J Reprod Med 1979;23:228.
11. Garite TJ, Freeman RK, Nageotte MP. Fetal maturity cascade: a rapid and cost effective method for fetal lung maturity testing. Obstet Gynecol 1986;67:619.
12. Maniscalco WM, Finkelstein JN, Parkhurst AB. De novo fatty acid synthesis in developing lung. Biochim Biophys Acta 1982;711:49.
13. Groener JEM, Van Rozen AJ, Cibelens DW. Cholesteryl ester transfer activity: localization and role in distribution of cholesteryl ester among lipoproteins in man. Atherosclerosis 1984;50:261.

Evaluation of serum CA 125 levels in the monitoring of ovarian cancer

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Serum CA 125 levels were evaluated in 227 patients with ovarian cancer. CA 125 levels were elevated in 86% of the patients. All histologic types, including mucinous tumors, were associated with raised CA 125 levels. There was a positive correlation with tumor burden and an inverse correlation with degree of differentiation. In patients undergoing radical operation an elevated CA 125 level was a bad prognostic index. Serial CA 125 measurements were assessable in 112 patients undergoing chemotherapy. Rising or falling levels correlated with disease in 92% of the cases. The CA 125 level increased before clinical progression with a median lead time of 3 months. Only patients who showed objective response to chemotherapy had a decrease in antigen levels of ≥30% 4 weeks after the first course of chemotherapy and a normalization of CA 125 levels 3 months after initiation of chemotherapy. Rising levels were always associated with progression. These data suggest that CA 125 may aid in early identification of nonresponders. However, a normal CA 125 level does not exclude the presence of disease. (AM J OBSTET GYNECOL 1987;157:88-92.)

Key words: CA 125 serum levels, monitoring, ovarian cancer

The natural history of ovarian cancer is characterized by intra-abdominal spread, which hinders early diagnosis and accurate monitoring of the disease. During recent decades a large number of antigens have been detected in ovarian cancers. Bast et al.[1] developed a promising assay based on a murine monoclonal antibody (OC 125), reacting with the antigen CA 125, which is expressed by more than 80% of nonmucinous epithelial ovarian cancers. The objectives of this study were to investigate the prognostic value of preoperative and postoperative CA 125 serum levels in ovarian cancer, to determine the value of CA 125 in monitoring the disease, and to assess the correlation between CA 125 serum levels and histologic type and degree of differentiation.

Material and methods

A total of 606 serum samples from 227 patients with ovarian cancer have been tested for CA 125. The samples were obtained between February 1983 and February 1985 and stored at −20° C until they were analyzed. All patients had follow-up until July 1986. The results of the CA 125 test were not known at the time of treatment.

Clinical staging was performed according to the sys-

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Table I. Preoperative and postoperative CA 125 serum levels in ovarian cancer patients

		CA 125 level		
	n	*>35 U/ml (n)*	*Median (U/ml)*	*Range (U/ml)*
Preoperative	37	34	439	10-30800
Postoperative*				
Without residual tumor	57	9	18†	2-203
<2 cm Residual tumor	10	2	24†	6-1607
≥2 cm Residual tumor	46	42	187	6-18400

*Samples were obtained 3 to 6 weeks after primary operation and before the start of chemotherapy.

†$p < 0.001$ compared with the preoperative group and the postoperative group with ≥2 cm of residual tumor.

tems adopted by the international Federation of Gynecology and Obstetrics.[2] This series included 71 patients in Stage I, 13 in Stage II, 108 in Stage III, and 35 in Stage IV disease. The principles of therapy consisted of primary operation followed by cytotoxic chemotherapy with cisplatin, cyclophosphamide, Thiotepa, or carboplatin or by instillation of radioactive phosphorus.

Evaluation of response to treatment was based on clinical observations and radiologic data. Response to treatment was evaluated according to the recommendations of the World Health Organization.[3] Registration of disease progression requires a 25% or more increase in the size of the existing lesions or the appearance of a new lesion. Disease regression requires at least a 50% decrease in tumor size for at least 4 weeks for partial response and a disappearance of all clinical signs of malignancy for complete response. All relapses were confirmed by histologic or cytologic findings.

One of the authors (V. M. A.) reviewed all the slides. The results of the CA 125 investigation were not known at that time. Histologic classification was performed with the criteria defined by the World Health Organization.[4] One hundred sixteen tumors were serous, 31 mucinous, 22 endometrioid, 12 clear cell, 12 undifferentiated, 18 mixed, and 19 unclassifiable carcinomas. One patient had a malignant Brenner tumor, and three had nonepithelial tumors.

CA 125 assays. CA 125 was measured by an immunoradiometric ("sandwich") assay (Abbott CA 125 radioimmunoassay, Abbott Laboratories, North Chicago, Illinois), performed according to the instructions of the manufacturer. This kit uses antibody OC 125 bound to polystyrene beads and radiolabeled OC 125 to measure the amount of antigen bound from samples and standards during incubation. The interassay coefficient of variation was 6% with the control sera of the kit. The reference limit of 35 U/ml, as proposed by Bast et al.,[1] was verified in a small reference population. This group consisted of 45 patients with benign diseases. Only one patient in this group had a slightly elevated value. In serial analysis a doubling or halving of the CA 125 levels was considered a significant increase or decrease.

Sampling. Serum samples were obtained at the moment of referral to The Norwegian Radium Hospital. Most patients are referred in the postoperative phase or when they develop a relapse. This explains why we were unable to study the CA 125 levels in all patients during the entire treatment period.

Serum samples were available from 37 patients before staging laparotomy and from 113 patients after operation. Sampling was performed in the week before laparotomy in the former group and 3 to 6 weeks after operation and before the start of chemotherapy or radiotherapy in the latter group. The postoperative group consisted of 56 patients with residual disease and 57 patients without any. The group of patients with preoperative measurements was small because the majority of patients were referred to our hospital after primary operation.

In 112 patients with epithelial cancer serial measurements were performed during chemotherapy. Thirteen cases were excluded (11 with initially normal CA 125 levels and two with nonepithelial tumors). Serum samples were assessable at the moment of relapse in 29 patients who previously had received chemotherapy but had been free of treatment for at least 3 months.

Second-look laparotomy was performed in 21 patients without clinical or radiologic evidence of disease. Eleven patients were free of tumor after the primary operation. Serum samples were collected in the week before second-look operation.

Statistical analysis. For statistical analysis the χ^2 test and the Wilcoxon test were used.[5] When no statistical analysis is mentioned, the Wilcoxon test was used.

Results

CA 125 levels in preoperative and postoperative patients. Table I summarizes the preoperative and postoperative serum CA 125 levels. Postoperative CA 125 levels were significantly lower in patients without residual tumor and in patients with <2 cm of re-

Table II. Serum CA 125 levels in ovarian cancer patients with evidence of disease* in relation to histologic type

Histologic type	n	CA 125 level >35 U/ml (n)	Median (U/ml)	Range (U/ml)
Epithelial tumors				
Serous cystadenocarcinoma	68	61	346	7-30800
Mucinous cystadenocarcinoma	4	3	47†	15-157
Endometrioid carcinoma	8	6	308	6-1727
Clear cell carcinoma	6	3	46‡	12-329
Mixed carcinoma	10	9	286	15-3316
Undifferentiated carcinoma	4	3	309	10-1000
Unclassifiable carcinoma	12	11	333	6-1607
Mixed mesodermal tumor	1	1	395	
Carcinosarcoma	1	1	48	
Total	114	98 (86%)	266	6-30800

*Preoperative and postoperative patients with evidence of disease at the moment of sampling and patients with untreated relapse. Patients who had more than one sample assessable have the first sample included.

†p = 0.02 compared with serous cystadenocarcinoma.

‡p = 0.015 compared with serous cystadenocarcinoma.

Table III. Serum CA 125 levels in ovarian cancer patients with evidence of disease* in relation to degree of differentiation

Differentiation	n	CA 125 level >35 U/ml (n)	Median (U/ml)	Range (U/ml)
Borderline	4	3	57†	7-157
Grade 1	8	5	53‡	14-358
Grade 2	26	24	242	30-4278
Grade 3	76	66	387	6-30800

*Preoperative and postoperative patients with evidence of disease and patients with untreated relapse.

†p = 0.027 compared with values for grade 2 and 3 lesions.

‡p = 0.007 compared with values for grade 2 and 3 lesions.

sidual disease compared with the two other groups (preoperative or postoperative with >2 cm of residual disease; for each group $p < 0.001$). There was no significant difference between the latter two groups or between the former two groups ($p = 0.15$ and $p = 0.57$, respectively).

In 34 of 37 patients preoperative CA 125 values were elevated. One patient with Stage I disease, 24 with Stage III, and nine with Stage IV had elevated CA 125 levels. Three patients with Stage III had values within the normal range (two serous and one undifferentiated carcinoma).

The prognostic value of preoperative CA 125 levels was evaluated in 36 patients with Stage III or IV disease. The CA 125 levels tended to correlate inversely with survival (median follow-up 26 months; $p = 0.06$).

The prognostic value of postoperative CA 125 levels (sampled 3 to 6 weeks after staging laparotomy) in patients without residual tumor was evaluated in 57 cases. A raised postoperative CA 125 serum level implied a bad prognosis, since four of eight patients with elevated levels had relapse, whereas only five of 49 patients with normal levels had relapse (median follow-up 24 months; χ^2, $p < 0.02$).

Histologic type and differentiation. The relation between histologic type and serum CA 125 levels in patients with evidence of disease is shown in Table II. CA 125 levels were significantly higher in serous cystadenocarcinomas than in mucinous and clear cell carcinomas ($p = 0.02$ and $p = 0.015$, respectively), but no significant difference was observed when serous cystadenocarcinomas were compared with the other epithelial tumors.

Grade 2 and 3 tumors showed higher CA 125 serum levels than borderline and grade 1 tumors ($p = 0.027$ and $p = 0.007$, respectively). There was no significant difference between the former two groups (Table III).

CA 125 monitoring profiles during chemotherapy. Serial measurements were performed during chemotherapy in 112 patients with epithelial cancer. These

Table IV. Variation in CA 125 levels with disease status in response to chemotherapy

	Disease status			
*Antigen level**	*Complete response*	*Partial response*	*Stable disease*	*Progression*
Decreased	16	28	3	2
Remained unchanged	0	0	13	3
Increased	0	0	0	36
Total	16	28	16	41

*Doubling or halving of CA 125 levels was considered a significant increase or decrease.

Table V. CA 125 levels* and findings at second-look laparotomy

		Completeness of primary operation	
Antigen level	*Second-look finding*	*Residual tumor*	*No residual tumor*
CA 125 <35 U/ml	Tumor	3	0
	No tumor	5†	11§
CA 125 ≥35 U/ml	Tumor	1	0
	No tumor	1‡	0
Total		10	11

*Samples were obtained in the week before second-look laparotomy.

†Three patients had a relapse.

‡Relapse.

§One patient had a relapse.

findings are presented in Table IV. CA 125 levels correlated with the clinical course in 93 of 101 cases (92%).

A rising CA 125 level was always associated with disease progression ($n = 36$). In 16 of these patients a significant increase in CA 125 was observed at least 1 month before clinical evidence of progression (1 to 8 months, median 3 months). Despite obvious clinical progression, two patients had decreasing CA 125 values (one serous and one clear cell carcinoma).

CA 125 values normalized in 14 of 16 patients with complete response. Twelve of the 14 patients had a relapse during the observation period. During partial remission, CA 125 values normalized in nine of 28 patients, whereas CA 125 levels remained elevated in all patients with stable or progressive disease. CA 125 measurements were performed 4 weeks after the first course of chemotherapy in 14 patients with partial or complete remission. All patients had a decrease in CA 125 levels ranging from 30% to 95% (mean 65%), whereas none of the patients with stable or progressive disease showed a decrease of ≥30%. Stable disease was noted in 16 patients. In 13 patients the CA 125 levels remained unchanged, whereas halving of the antigen level was observed in three patients.

Relapse. CA 125 levels were elevated in 27 of 29 patients who developed relapse. All patients with previously elevated CA 125 levels showed increasing antigen levels at the time of relapse.

CA 125 levels at second-look laparotomy. Second-look laparotomy was performed in 21 patients (Table V). The tumor sizes associated with a normal CA 125 level in patients with previously elevated antigen values ranged from 5 to 20 mm. One patient, who was free of tumor at second-look laparotomy but had an elevated CA 125 level, had an intraperitoneal relapse 16 months later.

Comment

CA 125 is an antigen associated with a high molecular weight glycoprotein, which is expressed by more than 80% of nonmucinous ovarian carcinomas.[1, 6-10] The specificity for ovarian carcinoma is poor, as the antigen is also associated with other carcinomas.[1, 6, 7, 10] Consequently, serum CA 125 has not proved useful for differential diagnosis of carcinomas of unknown origin. CA 125 has been shown to be elevated in a number of benign conditions such as endometriosis, pregnancy, pelvic inflammatory disease, and liver cirrhosis,[7, 10, 11] precluding use of CA 125 estimation as a screening method for asymptomatic patients. However, CA 125 has been shown to be promising for monitoring patients with epithelial ovarian cancer.[1, 6-9]

This study reveals an elevation of serum CA 125 levels in 86% of patients with ovarian cancer. This finding is consistent with earlier studies.[1, 6-9] Originally the usefulness of the test was restricted to nonmucinous epithelial tumors.[1] However, later reports and our findings suggest that all histologic types of ovarian cancers can be accompanied by elevated CA 125 levels[6, 8, 9] (Table II). Although mucinous and clear cell carcinomas often show elevated CA 125 levels, the antigen levels were significantly lower than in serous cystadenocarcinomas. No difference was observed when serous carcinomas were compared with the other types of epithelial tumors. Furthermore, an inverse relationship between serum CA 125 levels and the degree of differentiation was observed (Table III). The higher CA 125 levels in less differentiated tumors were not caused by a difference in tumor burden, since no correlation was observed between the degree of differentiation and tumor burden (p = 0.4).

A remarkably good correlation (90% to 93%) has been reported between disease evolution during chemotherapy and CA 125 levels.[1, 6-9] This correlation is confirmed in the current study (92%) (Table IV). However, no additional information would be obtained from this test if the data coincided with the clinical findings. It is therefore interesting to note that both in earlier studies and in the present report elevations of CA 125 preceded clinical detection of progression with a median of 3 months.[9, 12] In the current series we observed that rising CA 125 levels were always associated with progressive disease and that 4 weeks after the first course of chemotherapy all responding patients exhibited a 30% to 95% decrease in the antigen levels, whereas none of the patients with stable or progressive disease showed a decrease of ≥30%. Normalization of CA 125 3 months after the initiation of chemotherapy was observed only in patients with complete or partial response. These findings suggest that early identification of nonresponders, resulting in a reduction of toxicity by avoidance of ineffective therapy, is possible.

Sevelda et al.[6] suggested that chemotherapy may select CA 125–negative cell clones. This phenomenon has also been observed with other tumor markers.[13] We observed no such tendency, as all patients with previously elevated CA 125 levels showed rising values when developing a relapse after chemotherapy.

Several studies[12, 14-16] have demonstrated that an elevated CA 125 level may predict the presence of intraperitoneal tumors at second-look procedures but that a normal CA 125 level does not exclude the presence of disease. These findings were confirmed in the present study; the maximum tumor size associated with a normal CA 125 level in patients with previously elevated antigen concentrations was 20 mm. One patient free of tumor at second-look laparotomy but with an elevated CA 125 level had an intraperitoneal relapse 16 months later.

The prognostic value of preoperative and postoperative CA 125 levels has been evaluated in this study. The preoperative CA 125 levels tended to correlate inversely with survival in patients with Stage III or IV disease. In patients without residual tumor an elevated CA 125 level (sampled 3 to 6 weeks after operation) was a bad prognostic index.

REFERENCES

1. Bast RC, Klug TL, St. John E, et al. A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. N Engl J Med 1983;309:883.
2. Petterson F, Kolstad P, Ludwig H, et al. Annual report on the results of treatment in gynecologic cancer. Stockholm: Radiumhemmet, 1985, vol 19:210-3.
3. Miller AB, Hoogstraten B, Staquet M, et al. Reporting results of cancer treatment. Cancer 1981;47:207.
4. Serov SF, Scully RE, Sobin LH. Histological typing of ovarian tumours. In: International histological classification of tumours. no. 9. Geneva: World Health Organization, 1973:17-8.
5. Goldstein A. Biostatistics: an introductory text. New York: Macmillan, 1964.
6. Sevelda P, Salzer H, Dittrich C, et al. Die klinische Bedeutung des Tumormarkers CA-125 fur die praeoperative Diagnostik und die postoperative Nachbetreuung von Patientinnen mit malignen Ovarialtumoren. Geburtshilfe Frauenheilkd 1985;45:769.
7. Ricolleau G, Chatal JF, Fumoleau P, Kremer M, Douillard JY, Curtet C. Radioimmunoassay of the CA125 antigen in ovarian carcinomas: advantages compared with CA19-9 and CEA. Tumour Biol 1984;5:151.
8. Canney PA, Moore M, Wilkinson PM, James RD. Ovarian cancer antigen CA125: a prospective clinical assessment of its role as a tumour marker. Br J Cancer 1984;50:765.
9. Crombach G, Zippel H, Wurz H. Erfahrungen mit CA125, einem Tumormarker fur maligne epitheliale Ovarialtumoren. Gerburtshilfe Frauenheilkd 1985;45:205.
10. Hallilla H, Stenman UH, Seppalla M. Ovarian cancer antigen CA 125 levels in pelvic inflammatory disease and pregnancy. Cancer 1986;57:1327.
11. Barbieri RL, Niloff JM, Bast RC, Schaetzl E, Kistner RW, Knapp RC. Elevated serum concentrations of CA-125 in patients with advanced endometriosis. Fertil Steril 1986; 45:630.
12. Niloff JM, Knapp RC, Lavin PT, et al. The CA 125 assay as a predictor of clinical recurrence in epithelial ovarian cancer. AM J OBSTET GYNECOL 1986;155:56.
13. Vergote I, Onsrud M, Nustad K. Placental alkaline phosphatase as a tumor marker in ovarian cancer. Obstet Gynecol 1987;69:228.
14. Niloff JM, Bast RC, Schaetzl EM, Knapp RC. Predictive value of CA125 antigen levels in second-look procedures. AM J OBSTET GYNECOL 1985;151:981.
15. Atack DB, Nisker JA, Allen HH, Tustanoff ER, Levin L. CA 125 surveillance and second-look laparotomy in ovarian carcinoma. AM J OBSTET GYNECOL 1986;154:287.
16. Berek JS, Knapp RC, Malkasian GD, et al. CA 125 levels correlate with second-look operations among ovarian cancer patients: a prospective multi-institutional study. Obstet Gynecol 1986;67:685.

Serum copper level in gynecologic malignancies

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Serum copper level was determined before operation in 179 patients with various histologically proved gynecologic tumors: malignant, benign, or metastases to the ovary. Serum copper level was significantly higher ($p < 0.01$) in all groups of patients with cancer and in the benign group when compared with control subjects. Serum copper level correlated well with stage of cancer disease ($r = 0.70$ to 0.79) except for ovarian carcinoma, in which serum copper level was already significantly elevated in Stages I and II. The sensitivity of serum copper level >150 μg/dl in detecting malignancy was 87% to 100% in late cancer stages in all malignancies. Our data imply that the addition of serum copper level determination to other screening tests could increase their sensitivity. (AM J OBSTET GYNECOL 1987;157:93-6.)

Key words: Serum copper level, gynecologic malignancies

Serum copper levels have been found to be elevated in the presence of certain neoplastic processes and in some benign nonneoplastic conditions. The closest correlation between serum copper levels and the activity of the malignant process was shown in malignant lymphomas in general, in Hodgkin's disease in particular, and in acute lymphocytic leukemia.[1] High serum copper levels were reported in solid tumors such as carcinoma of the lung, gastrointestinal carcinoma, breast carcinoma, and osteosarcoma.[2, 3] Among gynecologic malignancies, serum copper levels were investigated only in carcinomas of the cervix and ovary.[4-6] We have previously reported that a serum copper level of 150 μg/dl clearly separates patients with a pelvic mass on the basis of ovarian carcinoma and those with benign noninflammatory pelvic lesions.[6]

The aim of this study was to assess the significance of serum copper levels as a tumor marker in different gynecologic malignancies. This study was carried out in a large group of patients with various histologically proved gynecologic malignancies and correlated serum copper levels with the stage of the disease.

Material and methods

All women admitted to the Gynecological Service of Hadassah University Hospital for the evaluation of a possible gynecologic malignancy during 4 consecutive years were studied. Patients known to have liver disease or those receiving estrogen or other hormonal therapy were excluded. Blood samples for copper determination were collected before any treatment was given to minimize the variations that might have resulted from the stress of the surgery. The final diagnosis was based on histologic proof in all patients. Staging was assigned according to the International Federation of Gynecologists and Obstetricians classification system. Twenty healthy women served as the control group.

Serum copper analysis. To avoid trace element contamination, blood was collected in specially cleaned plastic syringes and placed in plastic tubes until coagulation occurred. Serum was separated and deep frozen in another set of plastic tubes until the day of the test. Serum samples were diluted three times to avoid the problem of viscosity and aspiration rate. A permanent control (Monitor I lyophilized Dade division, United States) was used, and pooled serum was run as a secondary control. Titrisol standard solution (Merck Sharpe and Dohme, West Germany) was used as the stock standard, and a calibration curve was made from freshly prepared standard of 50, 100, 200, 300, and 400 γ% in pooled serum. Each test was performed in duplicate by the atomic absorption spectrophotometer (Varian Technicon Model 1200 atomic absorption spectrophotometer, Techtron, Melbourne, Australia). The optimal wave length was 324.7 mm with a 0.2 mm slit width. The recovery rate was 94% to 96%, with standard deviation ± 6.4%.

Statistical methods. Student *t* test and linear regression test were used for statistical evaluation of the results.

Results

One hundred seventy-nine patients and 20 control subjects were included in the final study. One hundred three patients had gynecologic malignancies (vulvar, nine; cervical, 22; endometrial, 25; and ovarian, 47),

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Table I. Serum copper levels in the various groups of patients according to stage of disease (number of patients)

		Serum copper levels ($\mu g/dl$) (mean ± SEM)				
			Stage			
Diagnosis	*No. of patients*	*All*	*I*	*II*	*III*	*IV*
Vulvar cancer	9	163 ± 10‡	(6) 148 ± 9†	—	(2) 181 ± 17§	(1) 215*‡
Cervical cancer	22	159 ± 10‡	(9) 129 ± 10‖	(5) 145 ± 8†	(5) 188 ± 20‡	(3) 266 ± 26‡
Endometrial cancer	25	152 ± 6‡	(18) 142 ± 5†	(3) 164 ± 7*‡	(2) 183 ± 14*‡	(2) 202 ± 34†
Ovarian cancer	47	185 ± 4*‡	(4) 174 ± 6*‡	(4) 189 ± 9*‡	(24) 184 ± 7*‡	(15) 189 ± 8*†
Nongynecologic cancers	14	200 ± 11*‡	—	—	—	(14) 200 ± 11‡
Benign lesions	62	145 ± 3§				
Controls	20	126 ± 4				

*$p < 0.05$ compared with benign lesion.
†$p < 0.05$ compared with the control group.
‡$p < 0.01$ compared with the control group.
§$p < 0.001$ compared with the control group.
‖Not significant compared with the control group.

14 patients had nongynecologic cancers, and 62 patients had benign lesions. Table I summarizes the number of patients (subgrouped according to the International Federation of Gynecologists and Obstetricians clinical stage of the disease) in each diagnosis with the corresponding serum copper level. The serum copper level was significantly higher ($p < 0.01$) in all groups of patients with cancer and in the benign group when compared with the control group. The mean serum copper level was not significantly different when compared with the benign group in all stages of gynecologic cancers except for all patients with ovarian carcinoma. In patients with ovarian carcinoma, regardless of the stage of the disease, the serum copper level was statistically different when compared with that found in the benign group ($p < 0.05$). In general, a higher mean serum copper level was noted in all subgroups with malignancy in Stages III and IV when compared with the benign group, but in some patients this was of no statistical significance since the number of patients was small.

Serum copper levels correlated with the stage of cancer disease in all gynecologic malignancies except for ovarian carcinoma in which serum copper levels were already significantly elevated in Stages I and II. Fig. 1 illustrates this correlation. All nongynecologic malignancies were in Stage IV. They consisted of seven patients with colon carcinoma, two with pancreatic carcinoma, three with metastatic breast carcinoma, and two with dessiminated malignant melanoma. The highest serum copper levels of all patients in our study were found in this group (200 ± 11 μg/dl). Sixty-two patients were diagnosed as having benign gynecologic lesions such as myoma uteri, ovarian cyst, or cervical polyp. Twenty three of these patients (37%) had a serum copper level >150 μg/dl. Six of these patients with a serum copper level >150 μg/dl had torsion of an ovarian cyst or myoma with necrosis, five had a benign lesion and had had cholecystectomy, four suffered from congestive heart failure and were treated with digoxin and diuretics, and one had severe anemia. In seven patients, no suggestive cause for the elevated serum copper level was found.

The sensitivity of serum copper levels >150 μg/dl in detecting malignancy in the various subgroups of patients is outlined in Table II. It is apparent that the sensitivity is significantly higher in late cancer stages in all malignancies (87% to 100%) when compared with the sensitivity in early cancer stages (21% to 43%) except for ovarian carcinoma, in which it detected all eight patients in Stages I and II. Only two of 47 patients with ovarian carcinoma were not detected by this criterion of serum copper levels.

Comment

Significantly elevated levels of serum copper were demonstrated in this study in patients with both gynecologic and nongynecologic cancers. Not only is this test highly sensitive, especially in disseminated cancer, but the level also correlated with the stage of the disease ($r = 0.70$ to 0.79). Such correlation was clearly shown in malignant lymphomas[1] but has yet to be shown in gynecologic malignancies. In a previous study[6] we reported that a serum copper level of 150 μg/dl separated patients with ovarian carcinoma from those with benign noninflammatory pelvic lesions. We also reported that serum copper levels may be used to monitor the efficacy of chemotherapy; elevated serum copper levels in patients with ovarian carcinoma decreased significantly as the disease activity was reduced in response to therapy and indicated a remission state. Others[4] have reported an elevation in serum copper levels in gynecologic ma-

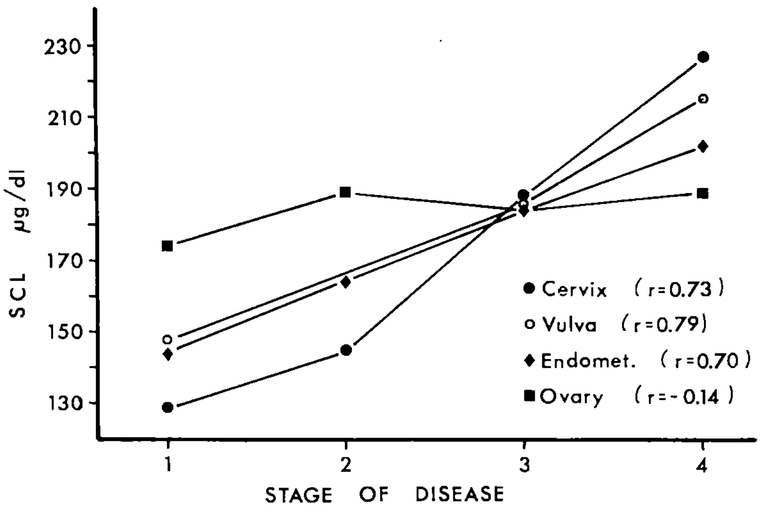

Fig. 1. Serum copper levels at the various stages of gynecologic cancers.

Table II. The sensitivity of serum copper levels >150 μg/dl in detecting malignancy in various groups of patients

Diagnosis	*N*	*Stages I + II (%)*	*N*	*Stages III + IV (%)*	*N*	*All (%)*
Vulvar cancer	6	33	3	100	9	55
Cervical cancer	14	21	8	87	22	45
Endometrial cancer	21	43	4	100	25	52
Ovarian cancer	8	100	39	95	47	96
Nongynecologic cancers	—	—	14	93	14	93
Total	49	45	68	94	117	73

lignancies but in very small series. Correlation with the stage of disease was reported only in cervical cancer.[5] Outstanding in this regard in our study[6] is ovarian carcinoma, in which serum copper levels were significantly higher than the healthy state already in Stages I and II. Ovarian carcinoma is also notable for the high sensitivity of the serum copper level test for its detection. Forty-five of 47 patients with ovarian carcinoma were detected by serum copper levels >150 μg/dl with 96% sensitivity. It is of interest to note that serum copper levels in ovarian carcinoma were not only elevated when compared with the healthy state but also when compared with serum copper levels in patients with benign lesions. The elevation in serum copper levels already evident in early stages of ovarian carcinoma may be attributed to elevated estrogen levels as seen in pregnancy or contraceptive treatment.[7] Scully[8] stated that patients with primary epithelial tumors of the ovary have enhanced endocrine activity of the stroma. According to the author ovarian tumors secrete hormones and especially estrogens at subclinical levels. A negative result of the serum copper level test (according to the 150 μg/dl level) in patients with a pelvic mass strongly indicates that ovarian carcinoma is not the cause and also that pelvic metastases from nongynecologic malignancy could be ruled out. Furthermore, a low serum copper level in gynecologic malignancy other than ovarian carcinoma indicates a localized disease.

The exact mechanism of hypercupremia in malignancy is unclear. Some authors[9] have raised the theory that the increase in serum copper levels may be a defense mechanism against tumors, since copper can retard the growth of tumors in vitro or inhibit their development in vivo. Others[10] hypothesize that ceruloplasmin is resialylated at the tumor cell surface and the decrease in catabolism, which is due to this process, could account for the increase in serum copper levels in patients with neoplasia. Moreover, an increase in serum hexosamine, which is brought about by the release of tumor degradation products, was found to be positively correlated with a rise in serum copper levels and has been considered a possible mechanism.[11]

When serum copper levels are considered an additive to the long list of cancer screening tests, one should be cautious about overly relying on this test. Numerous nonmalignant conditions can cause an elevation in serum copper levels, such as nonspecific inflammatory processes, liver disease, pregnancy, and estrogen treatment. In certain conditions the cause for the elevation remains unknown. One of the 20 healthy women in our control group had a serum copper level >150 μg/dl level. Twenty three of the 62 patients with benign gynecologic lesions (37%) had serum copper levels >150 μg/dl. Inflammatory processes, congestive heart failure, and anemia could probably account for this elevation in 11 patients. The other 12 patients had no apparent cause. It is tempting to relate the elevation in serum copper levels with the state after cholecystectomy in five patients, since most of the copper is excreted from the body via the bile.

The problem of early detection of ovarian carcinoma was dealt with by Goswamy et al.,[12] who suggested that ultrasonography could be the modality of screening. Our data imply that the addition of serum copper level determination to this or any other screening test could increase the sensitivity.

The correlation found in this study between serum copper level and stage of disease is important not only from the diagnostic point of view but may also imply that theories on copper as one of the causes of cancer[13] should be reevaluated and elevation in serum copper levels should be regarded as a sequelae of cancer rather than its cause.

REFERENCES

1. Hrgovcic M, Tessmer CF, Thomas FB, Fuller LM, Gamble JF, Shullenberger CC. Significance of serum copper levels in adult patients with Hodgkin's disease. Cancer 1973; 31:1337-45.
2. Linder MC, Moor JR, Wright K. Serum ceruloplasmin and cancer. J Natl Cancer Inst 1981;67:264-74.
3. Fisher GL, Byers VS, Shifrine M, Levin AS. Copper and zinc levels in serum from human patients with sarcomas. Cancer 1976;37:356-63.
4. Birdi A, Gupta S, Gambhir SS. Serum copper in carcinoma of cervix uteri as an indicator of successful radiotherapy. J Surg Oncol 1983;22:193-6.
5. Brandes JM, Lightman A, Drugan A, Zinder O, Cohen A, Itskovitz J. The diagnostic value of serum copper/zinc ratio in gynecological tumors. Acta Obstet Gynecol Scand 1983;62:225-9.
6. Margalioth EJ, Udassin R, Maor J, Schenker JG. Serum copper level in ovarian carcinoma. Cancer 1985;56: 856-9.
7. Schenker JG, Jungreis E, Polishuk WZ. Serum copper levels in normal and pathologic pregnancies. AM J OBSTET GYNECOL 1969;105:933-7.
8. Scully RE. Ovarian tumors with estrogenic manifestations. Contemp Ob/Gyn 1977;10:83-7.
9. Linder MC. Iron and copper metabolism in cancer as exemplified by changes in ferritin and ceruloplasmin in rats with transplantable tumors. Adv Exp Med Biol 1977;92:643-64.
10. Kessel D, Allen J. Elevated plasma sialyltransferase in the cancer patient. Cancer Res 1975;35:670-2.
11. Tani P, Kokkola K. Serum iron, copper and iron binding capacity in bronchogenic pulmonary carcinoma. Scand J Respir Dis 1972;80(suppl):121-8.
12. Goswamy RK, Campbell S, Whitehead MI. Screening for ovarian cancer. Clin Obstet Gynecol 1983;10:621-41.
13. Margalioth EJ, Schenker JG, Chevion M. Copper and zinc levels in normal and malignant tissues. Cancer 1983; 52:868-72.

Placental glycolysis and energy metabolism in preeclampsia

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We examined the possibility that in preeclampsia complicated by fetal growth retardation, placental energy state is low either because of impaired glycolysis or because of ischemia resulting from reduced maternal placental blood flow. Concentrations of pyruvate and lactate, but not of glycogen and glucose, were significantly low in placentas of mothers with severe preeclampsia, supporting previous indirect evidence of inhibited glycolysis. Nevertheless, direct measurements of adenine nucleotide concentrations did not indicate reduced placental energy level in the preeclamptic placentas. This along with a lack of change of the ratio of lactate/pyruvate concentration (an indication of the redox state of cytoplasmic reduced nicotinamide adenine dinucleotide/nicotinamide adenine dinucleotide) is also evidence against the hypothesis of general placental ischemia leading to energy deficiency. However, as glycolysis is an important source of precursors, particularly pyruvate, for synthesis of amino acids and lipids, these results suggest that there is a significant metabolic abnormality in placentas of mothers with severe preeclampsia. (AM J OBSTET GYNECOL 1987;157:97-101.)

Key words: Placental glycolysis in preeclampsia; placental energy state in preeclampsia; placental ischemia in preeclampsia

The causes of the fetal growth retardation that occurs in preeclamptic toxemia are unknown. It is known that maternal placental blood flow in preeclamptic toxemia is reduced,[1, 2] and this is probably, at least in part, due to increased incidence of placental infarction and pathologic changes of the uterine spiral arteries that have been observed by light and electron microscopy.[3, 4] It has been suggested that this reduced flow impairs nutrient supply to the fetus. However, a lack of clear correlation between reduced intervillous blood flow rate and infant birth weight in severe preeclampsia[2] argues against this as a primary factor. Furthermore, there is as yet no evidence that nutrients or other substances whose placental transfer is blood-flow limited (notably the blood gases and perhaps glucose) become limiting factors in fetal growth in these circumstances.

Another possibility is that placental function is itself impaired. Two recent lines of investigation suggest this possibility in different ways. We have found that in normal human placentas tissue energy level falls rapidly during experimental ischemia apparently because there is no appreciable stimulation of anaerobic adenosine triphosphate synthesis through the glycolytic pathway.[5] This indicates that the placenta may be vulnerable to reduced blood flow, and we have suggested that in preeclampsia a decreased energy state brought about by ischemia could compromise energy-dependent placental functions such as active placental transfer (e.g., of the amino acids) and protein hormone synthesis.[5] Somewhat in accord with this, structural and ultrastructural abnormalities attributable to ischemia, including focal areas of necrosis in the syncytiotrophoblast, have been observed in tissue of placentas from preeclamptic pregnancies.[6] Necrotic and prenecrotic areas in placentas of pregnancies complicated by intrauterine growth retardation (both with preeclamptic toxemia and without) have recently been identified as located in syncytiotrophoblast immediately adjacent to occluded or partially occluded spiral arteries.[4] It is possible that these areas of necrosis are the visible foci of more general ischemia. If this hypothesis is correct we would expect placentas from pregnancies with severe preeclamptic toxemia and fetal growth retardation to show signs of general hypoxia and reduced energy level.

In the second line of investigation Diamant and Kissilevitz[7] found that the activity of pyruvate kinase, a rate-limiting enzyme of the glycolytic pathway, was low in placentas from pregnancies with severe preeclamptic toxemia and fetal growth retardation. This might be expected to reduce the flux through the glycolytic pathway and thus reduce anaerobic adenosine triphosphate synthesis. Besides possibly reducing cellular energy level, inhibition of glycolysis would also be

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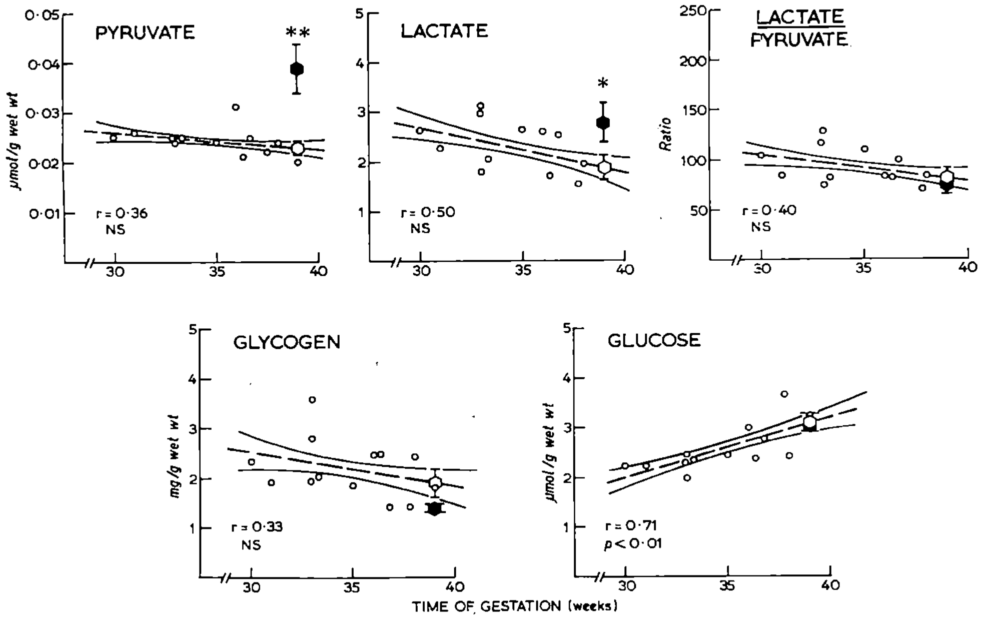

Fig. 1. Glycolytic metabolite concentrations in preeclamptic and normal placentas, by gestational age. ○, Individual values from 13 preeclamptic placentas. The regression (*broken lines*) are shown with the standard errors about them (*continuous lines*). ○ , Mean values from preeclamptic placentas at 39 weeks' gestation, computed by linear regression analysis from the individual values.[8] ● , Mean values from eight control placentas at 39 weeks. *Vertical bars* indicate the standard error of the mean. For lactate and lactate/pyruvate ratio a 39-week preeclamptic value is hidden by the mean value symbol. ** = $p < 0.01$; * = $p < 0.05$.

expected to alter the concentration or availability of certain key metabolites. One of these is pyruvate, which is the main substrate for the citric acid cycle (hence, aerobic adenosine triphosphate synthesis) and a central intermediate in lipid and amino acid as well as carbohydrate metabolism (see Comment). However, pyruvate kinase is under complex metabolic control, and a reduced assayed total pyruvate kinase activity would be meaningful only if it reflected an in vivo change that does bring about an inhibition of glycolysis and does affect the concentration of pyruvate or other key metabolites in preeclampsia.

We report here an initial study to test this by measuring the concentrations of the immediate products of glycolysis and of pyruvate kinase itself (pyruvate and lactate) as well as the substrates of glycolysis (glycogen and glucose) in placentas from mothers with severe preeclamptic toxemia, and comparing them with concentrations from normal mothers. In addition, to test whether there is a general ischemia of placental tissue in preeclampsia, we searched for evidence of tissue hypoxia in noninfarcted areas by measuring the lactate/pyruvate concentration ratio, which is an indication of the redox state of the cytoplasmic reduced nicotinamide adenine dinucleotide/nicotinamide adenine dinucleotide couple.[5] Also, as both hypotheses involve the possibility of an effect on placental energy state, we have examined this directly by measuring the concentrations of the individual adenine nucleotides in both groups of placentas.

Material and methods

Experimental design. Labor and vaginal delivery affect both adenine nucleotide and glycolytic metabolite concentrations in human placenta.[5] For this reason the study was confined to placentas delivered by cesarean section. As most patients with severe preeclamptic toxemia were delivered of their infants prematurely, it was not possible to obtain gestation-matched controls from normal pregnancies. Therefore, to allow for any effect of gestational age, values from preeclamptic placentas were subjected to regression analysis to compute an estimated mean value and standard error at 39 weeks.[8] This was then compared by conventional t test with values from a group of normal placentas sampled during cesarean section at 39 weeks.

Patients. There were 13 women with preeclamptic toxemia and eight unmatched controls. Control mothers were delivered of their babies by cesarean section for breech presentation, for cephalopelvic disproportion, or because of previous cesarean section. In none was there any placental problem, hypertension, or antenatal complication. The study subjects were inpatients at Queen Charlotte's Maternity Hospital and had preeclampsia as defined by The American College of Obstetricians and Gynecologists.[9] Ten of the patients with

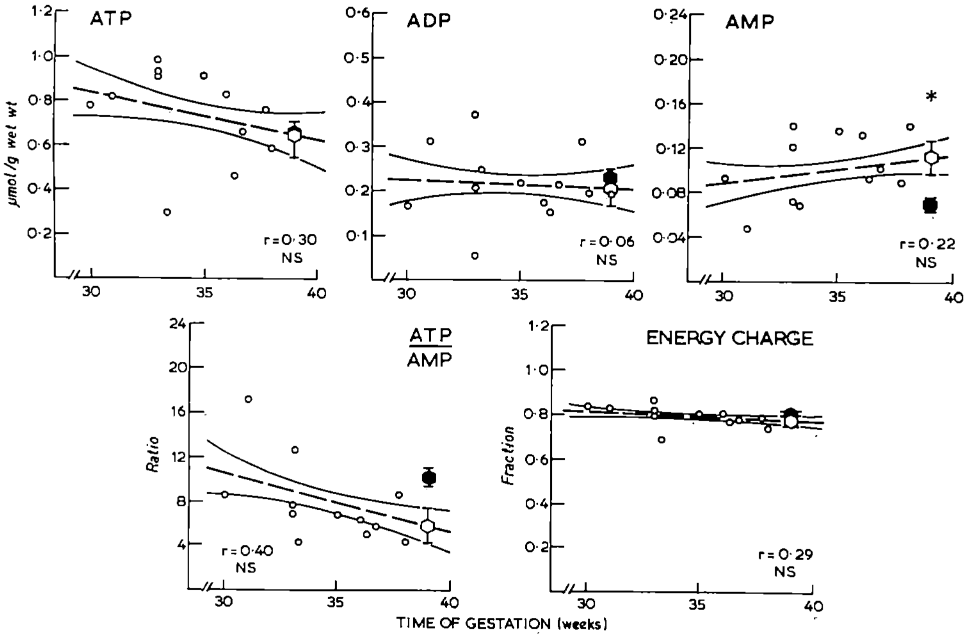

Fig. 2. Adenine nucleotide concentrations in preeclamptic and normal placentas, by gestational age. o, Individual values from preeclamptic placentas; ○ , computed 39-week mean values from preeclamptic placentas; ● , mean 39-week values from control placentas. Details as for Fig. 1. For adenosine triphosphate *(ATP)*, adenosine monophosphate *(AMP)*, adenosine triphosphate/adenosine monophosphate ratio, and "energy charge," a 39-week preeclamptic value is hidden by the mean value symbol. * = $p < 0.05$. The difference for adenosine triphosphate/adenosine monophosphate ratio between preeclamptic and control values approaches significance ($t = 2.0$, $p \approx 0.05$). "Energy charge" values (Atkinson[10]) are given by ([ATP] + ½ [ADP])/([ATP] + [ADP] + [AMP]), where ADP = adenosine diphosphate.

preeclamptic toxemia (compared with three of the controls) were primiparous. The maximum diastolic blood pressure was 110 to 135 mm Hg in nine of the study group patients and 95 to 105 mm Hg in the other four. Five had proteinuria greater than 900 mg/24 hr. The highest antenatal uric acid level was 0.40 to 0.60 mmol/L in eight patients and 0.30 to 0.39 mmol/L in five. The indication for cesarean section was deterioration of fetal or maternal state in all cases. The gestational ages at delivery (mean, 35 weeks) can be seen in Figs. 1 and 2. Only two of the infants in the preeclamptic toxemia group weighed more than 2.5 kg, whereas eight weighed less than 2.0 kg and three less than 1.5 kg. The mean birth weight (1.95 ± 0.15 [SEM] kg) was significantly ($p < 0.001$) less than that of the controls (3.31 ± 0.14 kg), and seven of the 13 had intrauterine growth retardation (birth weight less than the tenth centile for gestational age). The mean age of the mothers in the two groups was similar (preeclamptic toxemia, 29 years; control, 32 years). We conclude from the clinical and laboratory data that the study group was composed of women with severe preeclamptic toxemia.

Placental tissue sampling. Placentas were delivered at cesarean section. In all but three cases section was performed under epidural blockade with 0.5% w/v bupivacaine hydrochloride (Marcaine, Duncan Flockhart, London, United Kingdom) or 1.5% w/v lidocaine hydrochloride (Xylocaine, Astra Chemicals, Watford, United Kingdom) or both. Three of the operations were performed with the patients under general anesthesia with halothane (Fluothane, I.C.I. Pharmaceuticals, Macclesfield, Cheshire, United Kingdom)/nitrous oxide/oxygen. No effect of different anesthetic regimen could be detected from the results.

For each placenta an area of villous tissue free of obvious infarcts or other damage was freeze-clamped with tongs precooled in liquid nitrogen, as soon as possible after delivery (41 ± 5 seconds for the study group and 39 ± 2 seconds for the controls). The frozen samples were stored in liquid nitrogen for later assay.

Metabolite assays. Placental tissue concentrations of pyruvate, lactate, glucose, glycogen, adenosine triphosphate, adenosine diphosphate, and adenosine monophosphate were all measured by spectrophotometric and fluorometric enzymatic methods as described in detail elsewhere.[5]

Results

Glycolytic metabolites. Fig. 1 shows that the estimated 39-week pyruvate and lactate concentrations in villous tissue of placentas from mothers with severe preeclamptic toxemia were significantly lower than in the 39-week control placentas. The ratio of lactate/pyruvate concentration was not significantly different. On the other hand, neither glycogen nor glucose concentration was reduced in the preeclamptic placentas; if anything, the glycogen concentrations tended to be somewhat (although not significantly) higher than in the controls.

Adenine nucleotides. The concentrations of adenosine triphosphate and adenosine diphosphate and the "energy charge"[10] were not significantly altered in tissue of placentas from the preeclamptic women (Fig. 2), but the 39-week adenosine monophosphate value was significantly higher, and the adenosine triphosphate/adenosine monophosphate ratio, as expected, tended to be lower than in normal placentas.

Comment

For the preeclamptic placentas the necessity to correct the measured results statistically for gestational age resulted in increased standard errors (See Figs. 1 and 2). Even so, metabolite concentrations were found to be significantly different from those in controls.

The reduced tissue concentrations of pyruvate and lactate, the products of glycolysis (Fig. 1), without a reduction in the concentrations of the precursors glycogen and glucose indicate inhibition of a step in the glycolytic pathway in the preeclamptic placentas. This supports the finding by Diamant and Kissilevitz[7] of a lowered in vitro activity of pyruvate kinase and indicates that there is indeed a metabolic change in vivo. If these results reflect a reduced flux through the glycolytic pathway, they may also be consistent with a report that glucose utilization was reduced in placentas from deliveries of growth-retarded infants.[11] However, it was not stated whether these were associated with maternal preeclamptic toxemia. As pyruvate is a major substrate for the citric acid cycle, its reduced concentration could perhaps also explain the tendency toward low oxygen consumption reported by Tremblay et al.,[12] although, if flow through the citric acid cycle was reduced in our preeclamptic placentas, it did not seriously impair the tissue energy state (Fig. 2 and see below). On the other hand, Ginsburg and Jeacock[13] found a slightly greater production of lactate by incubated placental slices from preeclamptic pregnancies compared with normal pregnancies. This is inconsistent with our results but may represent an in vitro effect of the higher glycogen concentrations that we find in the preeclamptic placentas (Fig. 1).

An inhibition of the glycolytic pathway might itself have been expected to reduce cellular energy level in the preeclamptic placentas, but judged by adenosine triphosphate and adenosine diphosphate concentrations and "energy charge" (Fig. 2), this was not so. This lends support to our contention from previous work[5, 14] that the glycolytic pathway to lactate is not a major source of energy in the human placenta. Perhaps the role of glycolysis in energy production in this tissue is less important than its role in providing precursors for the synthesis of amino acids, lipids, nucleic acids, and other end products. This may be why placental lactate production continues even in conditions favorable for oxidative phosphorylation and is barely stimulated by ischemia or hypoxia.[5, 14]

Pyruvate (in tissues studied) is an important precursor generated by the glycolytic pathway, and pyruvate kinase is a key enzyme regulating the areas of metabolism mentioned above, at least in part through its influence on the availability of pyruvate.[15] Metabolic effectors of carbohydrate and lipid metabolism, including acetyl coenzyme A and citrate, are also formed from pyruvate, which is itself an activator of pyruvate dehydrogenase (which converts pyruvate to acetyl coenzyme A).[15] Therefore, if the role of pyruvate in human placenta is comparable with that in other tissues such as liver or adipose tissue—and enzyme patterns indicate that this is so[7]—then a reduction of its concentration would indicate a significantly altered placental metabolism.

Our results do not indicate at what stage in the progress of preeclampsia this metabolic abnormality develops. If it occurs early it is more likely to be of consequence to the fetus. It seems improbable that the rather specific changes that we find (see also below) are a part of the late general deterioration of the fetoplacental unit in fulminant preeclampsia.

Ischemia and energy state. Tissue hypoxia and ischemia generally elevate the ratio of lactate/pyruvate concentration because of an increase of the cytoplasmic reduced nicotinamide adenine dinucleotide/nicotinamide adenine dinucleotide ratio.[5] The lack of effect of preeclampsia on the ratio (Fig. 2) indicates that there was no appreciable general hypoxia of the cells. Neither was there a significant deficiency of adenosine triphosphate concentration or energy charge nor a raised adenosine diphosphate level to suggest impairment of placental energy state in the preeclamptic placentas. These results are not consistent with the hypothesis that placental insufficiency in preeclampsia is attributable to generally reduced energy level resulting from ischemia. Presumably any of the focal ischemic changes that have been observed microscopically[4, 6] affected a relatively small proportion of the tissue we studied and did not appreciably alter average adenine nucleotide concentrations. We did, however, avoid sampling obviously infarcted areas (see Material and methods).

Changes in cellular adenosine triphosphate concentration tend to be accompanied by much larger reciprocal percentage changes in adenosine monophosphate concentration because of the amplifying effect of adenylate kinase.[16] We did find significantly higher adenosine monophosphate concentrations in placentas from mothers with preeclampsia (Fig. 2). This may be an indication of a relatively early effect on adenosine triphosphate synthesis that was not detected by our measurements. In any case the high adenosine monophosphate concentration and tendency toward low adenosine triphosphate/adenosine monophosphate ratio (Fig. 2) in preeclamptic placentas is a further indication of altered metabolic state, particularly because both these nucleotides are themselves metabolic regulators.[16]

Whereas it is accepted that there are focal areas of structural change in preeclamptic placentas,[4,6] we could find no evidence of a generalized reduction of energy state, or ischemia, in the placentas we studied. We did, however, find evidence of an impairment of the glycolytic pathway, with reduced pyruvate and lactate concentrations in preeclamptic placentas. This may indicate a deficiency in the metabolic pathways of nutrient synthesis in these placentas, contributing to the state of "placental insufficiency" in established preeclampsia.

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REFERENCES

1. Dixon HG, McClure Brown JC, Davey DA. Choriodecidual and myometrial blood flow. Lancet 1963;2:369-73.
2. Kaar P, Jouppila P, Kuikka J, Luotola H, Toivanen J, Rekonen A. Intervillous blood flow in normal and complicated late pregnancy measured by means of an intravenous ^{133}Xe method. Acta Obstet Gynecol Scand 1980; 59:7-10.
3. Robertson WB, Brosens I, Dixon HG. Uteroplacental vascular pathology. Eur J Obstet Gynaecol Reprod Biol 1975;5:47-65.
4. Sheppard BL, Bonnar J. Ultrastructural abnormalities of placental villi in placentae from pregnancies complicated by intra uterine fetal growth retardation: their relationship to decidual spiral arterial lesions. Placenta 1980;1: 145-56.
5. Bloxam DL, Bobinski PM. Energy metabolism and glycolysis in the human placenta during ischaemia and in normal labour. Placenta 1984;5:381-94.
6. Fox H, Jones CJP. The ultrastructure of the placenta in maternal pre-eclampsia. In: Bonnar J, MacGillivray I, Symonds M, eds. Pregnancy hypertension. Lancaster: MTP Press, 1980:205-11.
7. Diamant YZ, Kissilevitz R. Placental metabolism in preeclamptic toxemia of pregnancy. In: Bonnar J, MacGillivray I, Symonds M, eds. Pregnancy hypertension. Lancaster: MTP Press, 1980:277-92.
8. Snedecor GW, Cochran WG. Statistical methods. Ames, Iowa: Iowa State University Press, 1956:135-40.
9. Hughes EC, ed. Obstetric-gynecologic terminology. Philadelphia: FA Davis Co, 1972.
10. Atkinson DE. The energy charge of the adenylate pool as a regulatory parameter. Interaction with feedback modifiers. Biochemistry 1968;7:4030-4.
11. Sybulski S, Tremblay PC. Pathways of glucose metabolism in human placentas from normal pregnancies and from pregnancies associated with intrauterine fetal malnutrition. AM J OBSTET GYNECOL 1969;103:1148-53.
12. Tremblay PC, Sybulski S, Maughan GB. Role of the placenta in fetal nutrition. AM J OBSTET GYNECOL 1965; 91:597-605.
13. Ginsburg J, Jeacock MK. Placental lactate production in toxemia of pregnancy. AM J OBSTET GYNECOL 1967; 98:239-44.
14. Bloxam DL. Human placental energy metabolism: its relevance to in vitro perfusion. Contrib Gynecol Obstet 1985;13:59-69.
15. Geelen MJH, Harris RA, Beynen AC, McCune SA. Shortterm hormonal control of hepatic lipogenesis. Diabetes 1980;29:1006-22.
16. Newsholme EA, Start C. Regulation in metabolism. London/New York: John Wiley & Sons, 1973.

Calculated capillary hydrostatic pressure in normal pregnancy and preeclampsia

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Capillary hydrostatic pressure has been calculated in normal pregnancy and preeclampsia. In humans, capillary hydrostatic pressure cannot be measured directly but may be calculated when the colloid osmotic pressure in plasma and interstitial fluid and interstitial fluid hydrostatic pressure are known (Starling equation). New methods have made it possible to measure the interstitial fluid colloid osmotic pressure and interstitial fluid hydrostatic pressure. In the present study interstitial fluid was collected from the subcutaneous tissue by implanted wicks (wick method), and interstitial fluid colloid osmotic pressure was determined. Interstitial fluid hydrostatic pressure was recorded by the wick-in-needle technique. Capillary hydrostatic pressure was calculated in 10 women in the first trimester and 10 in the third trimester of normal pregnancy, in 15 patients with mild preeclampsia, and in 13 women with severe preeclampsia. In normal pregnancy, capillary hydrostatic pressure increased by about 30% between the first and third trimesters. In mild preeclampsia, capillary hydrostatic pressure values did not differ significantly from those in the third trimester of normal pregnancy. However, in severe preeclampsia capillary hydrostatic pressure was significantly lower (40%) than in mild preeclampsia. Whether the low capillary hydrostatic pressure is caused by the severe general vasospasm seen in this condition or is a secondary event is unknown. (AM J OBSTET GYNECOL 1987;157:102-6.)

Key words: Pregnancy, preeclampsia, capillary pressure

The hemodynamic adjustment to pregnancy requires profound alterations in the maternal circulatory system. Some of the central hemodynamic changes have been studied extensively, but less attention has been given to changes in the microcirculatory system. As stated by Guyton,[1] the most purposeful function of the circulation occurs in the capillaries, namely, interchange of nutrients and cellular excreta between the tissues and the circulating blood.

As outlined by Starling,[2] the size and direction of capillary filtration are determined by the transcapillary differences in hydrostatic and colloid osmotic pressures across the capillary membranes and by the properties of the capillary wall. Any increase in interstitial fluid volume is caused by a net capillary filtration exceeding lymphatic flow (L). Net capillary filtration (F) is described by the equation:

$$F = CFC\,([Pc - Pi] - \sigma[COPp - COPi]) \quad \text{(Equation 1)}$$

where CFC, the capillary filtration coefficient, represents the amount of net filtrate formed in 100 gm of tissue/min for each rise in millimeters of mercury in net capillary filtration. Pc and Pi are average hydrostatic pressures in the capillaries and interstitial fluid. σ is the capillary reflection coefficient for plasma proteins. COPp and COPi are colloid osmotic pressures of plasma and interstitial fluid.

The present knowledge of transcapillary pressures in average capillaries is mainly based on experiments in laboratory animals. New methods, suitable for studies of the so-called "inaccessible tissue forces," i.e., interstitial fluid colloid osmotic pressure and interstitial fluid hydrostatic pressure, have made it possible to study transcapillary fluid balance in pregnant women.[3-5] When colloid osmotic pressure in plasma, interstitial fluid, and interstitial fluid hydrostatic pressure are known, the capillary hydrostatic pressure can be calculated. The purpose of the present investigation was to study changes in capillary hydrostatic pressure in normal pregnancy and in preeclampsia.

Material and methods

The following four groups were studied.

Normal pregnancy: First trimester. Ten healthy women who were 20 to 39 years old (mean, 29.8 years), pregnant in weeks 7 to 12 (mean, 8.4), were studied. The average systolic/diastolic blood pressure (mean ± SD) was 119 ± 7/77 ± 5 mm Hg.

Normal pregnancy: Third trimester. Ten women who were 23 to 35 years old (mean, 28.8 years), pregnant in weeks 30 to 40 (mean, 36.0), were studied. All the pregnancies were uneventful, and the birth weights

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Table I. Some characteristics of the four groups studied (means ± SD)

	Normal pregnant		*Preeclamptic*	
	First trimester (n = 10)	*Third trimester (n = 10)*	*Mild (n = 15)*	*Severe (n = 13)*
Blood pressure (mm Hg)	119 ± 7/77 ± 5	116 ± 13/75 ± 5	151 ± 8/99 ± 7	174 ± 10/115 ± 6
Proteinuria (gm/24 hr)	—	—	1.8 ± 1.3	6.4 ± 3.1
Hemoglobin (gm/dl)	13.0 ± 0.7	11.7 ± 1.1	12.5 ± 0.9	13.1 ± 1.0
Hematocrit (%)	38.4 ± 2.1	31.1 ± 2.3	37.6 ± 2.4	39.2 ± 2.4
Serum albumin (gm/L)	45.0 ± 1.8	36.2 ± 4.4	34.2 ± 4.1	27.2 ± 3.1
Serum total protein (gm/L)	68.8 ± 4.3	63.3 ± 4.5	59.8 ± 5.0	52.9 ± 4.1
Serum uric acid (mmol/L)	0.17 ± 0.03	0.25 ± 0.04	0.34 ± 0.06	0.41 ± 0.05

of all infants were appropriate for gestational age. The weight gain in pregnancy was 9 to 15 kg (mean, 12.9). The average blood pressure was 116 ± 13/74 ± 5 mm Hg. None of the subjects had clinical edema at the time of study.

Mild preeclamptic. Fifteen patients with mild preeclampsia (blood pressure ≥140/90 mm Hg and proteinuria >0.3 gm in 24-hour urine specimen) who were 18 to 33 years old (mean, 25.4), pregnant in week 35 to 39 (mean, 37.4) were studied. All the patients were nulliparas. The average blood pressure was 151 ± 8/99 ± 7 mm Hg and proteinuria was 1.8 ± 1.3 gm/24 hr. Weight gain in pregnancy was 7 to 25 kg (mean, 14.7). One of the patients was delivered by cesarean section. Birth weights were 2550 to 4650 gm (mean, 3147).

Severe preeclamptic. Thirteen patients with severe preeclampsia (blood pressure >160/110 mm Hg and proteinuria >3 gm/24 hr) who were 20 to 31 years old (mean, 26.2), pregnant in week 27 to 39 (mean, 32.8), were studied. All were nulliparas. The average blood pressure at the time of investigation was 174 ± 10/115 ± 6 mm Hg and proteinuria was 6.4 ± 3.1 gm/24 hr. Weight gain was 10 to 24 kg (mean, 18.7). Six of the patients were delivered by cesarean section. Birth weights were 950 to 3600 gm (mean, 2064).

All patients were hospitalized, and the studies were performed between 9.00 and 11.00 AM, after at least 9 hours of bed rest. None of the patients had any history of hypertension or kidney disease before pregnancy. Some laboratory characteristics of the patients studied are given in Table I. None had received any type of antihypertensive treatment. All patients were given full information about the experimental procedure before they gave their consent to take part in the study.

Plasma colloid osmotic pressure. During the experiment plasma was obtained from an antecubital vein by means of a loose and short-lasting venous stasis. Colloid osmotic pressure was measured on a colloid osmometer made for 5μl samples.[6]

Interstitial fluid colloid osmotic pressure. Fluid for determination of interstitial colloid osmotic pressure was sampled by means of the wick method as described earlier.[3, 4] Four to six nylon wicks were sewn into the subcutaneous tissue on the side of thorax at heart level and the same number on the lateral side of the lower leg. The skin was anesthetized by intradermal injection of approximately 0.1 ml lidocaine (Xylocaine, 20 mg/ml without adrenaline) where the needle was inserted and pulled out. The wicks (about 6 cm long) were left in place for 1 hour to equilibrate with the surrounding tissue fluid and then pulled out. The ends that protruded from the skin were carefully cut off. The remaining part of the wicks were immediately transferred to tubes containing mineral oil to avoid evaporation. Wick fluid was then isolated by centrifugation, and colloid osmotic pressure was determined in the colloid osmometer previously mentioned. One wick was usually enough to obtain a sample of at least 5μl that was needed for the measurement of interstitial fluid colloid osmotic pressure. Blood-stained wicks were discarded (15% to 20%). The working mechanism of the wicks has been thoroughly investigated, and it has been found that the colloid osmotic pressure of wick fluid gives a good estimate of colloid osmotic pressure in undisturbed interstitium.[8]

Interstitial fluid hydrostatic pressure. Interstitial fluid hydrostatic pressure in subcutaneous tissue was measured by the wick-in-needle technique.[4, 5] A steel cannula with an outside diameter of 0.6 mm was filled with a loose bundle of nylon filaments. The cannula was provided with a 3 to 4 mm long side-hole about 5 mm from the tip and connected to a pressure transducer (Statham model P23Db) by a polyethylene tube (Portex manometer line 60 cm, 200/490/060). The system was then filled with a sterile saline solution (9 mg/ml). After calibration, zero pressure was recorded in a 1 ml syringe filled with sterile saline solution and taped to the skin. The needle was then inserted into the subcutaneous tissue at the same level as the syringe and in the same areas in which the wicks were inserted. The hydrostatic pressure was then recorded.

Calculation of capillary hydrostatic pressure. In a

Table II. The colloid osmotic pressure of plasma (COP plasma), interstitial fluid on thorax (COPi thorax), and of the ankle (COPi ankle); interstitial hydrostatic pressure on thorax (Pi thorax) and at the ankle (Pi ankle) (means ± SD)

	Normal pregnant		*Preeclamptic*	
	First trimester (*n = 10*)	*Third trimester* (*n = 10*)	*Mild* (*n = 15*)	*Severe* (*n = 13*)
COP plasma (mm Hg)	23.2 ± 0.8	21.1 ± 1.2	19.8 ± 1.7	15.3 ± 1.7
COPi thorax (mm Hg)	13.1 ± 1.2	8.4 ± 0.8	6.8 ± 1.2	8.2 ± 1.3
COPi ankle (mm Hg)	9.6 ± 1.8	5.5 ± 1.4	3.9 ± 1.4	5.9 ± 2.2
Pi thorax (mm Hg)	−1.6 ± 0.7	−1.2 ± 0.9	−0.8 ± 1.1	−0.4 ± 1.0
Pi ankle (mm Hg)	−0.9 ± 0.5	−0.1 ± 0.4	0.7 ± 1.0	0.8 ± 1.1

situation with no net transcapillary filtration ($F = O$, Equation 1) and thereby no lymph flow, mean hydrostatic pressure in the capillaries is equal to the sum of all pressures opposing filtration (i.e., isogravimetric capillary pressure).[7] For a capillary exchange system, σ may theoretically vary from 0 to 1. If capillaries are freely permeable to proteins, σ equals 0 and if impermeable to proteins σ is 1. In subcutaneous tissue σ is probably close to 1.0.[8] Provided a reflection coefficient for plasma proteins of 1.0, capillary hydrostatic pressure can be determined as follows:

$$Pc = COPp - COPi + Pi \text{ (Equation 2)}$$

Since lymph flow cannot be less than zero, the calculated capillary hydrostatic pressure will be a minimum value.

Other measurements. Albumin, total protein, uric acid, and creatinine concentrations in serum were measured by an autoanalyzer. Protein concentration in urine was measured in 24-hour specimen with a Biuret method.

Statistics. The results were analyzed by the Student *t* test for unpaired and paired data with 5% as the level of significance.

Results

The colloid osmotic pressure in plasma and interstitial fluid and hydrostatic pressure in interstitial fluid are presented in Table II. The calculated capillary hydrostatic pressures will be given and are shown in Fig. 1.

Capillary pressure in normal pregnancy. Between the first and third trimesters a significant increase in calculated capillary hydrostatic pressure occurred both on thorax and at the ankle ($p < 0.005$). On thorax capillary hydrostatic pressure was 8.3 mm Hg (SD 1.9) in the first trimester and 11.5 mm Hg (SD 2.3) in the third trimester and 12.7 mm Hg (SD 2.1) and 15.5 mm Hg (SD 2.3), respectively, at the ankle.

Capillary pressure in preeclampsia. In the mild preeclamptic group, calculated capillary hydrostatic pressure on thorax was on average 12.2 mm Hg (SD 1.6) and 16.6 mm Hg (SD 1.0) at the ankle. Both values are slightly increased compared with the findings in the normal third-trimester group, but the difference is statistically not significant. In the severe preeclamptic group, capillary hydrostatic pressure on thorax was on average 6.7 mm Hg (SD 1.8) and 10.2 mm Hg (SD 2.8) at the ankle. Both values are significantly lower ($p < 0.001$) than in the mild preeclamptic group.

Comment

The capillary hydrostatic pressure has been measured directly in few tissues. In humans determination have been limited to the capillary loops in the nail bed.[1] The calculated capillary hydrostatic pressures in our studies are consistently lower than pressures measured directly. However, in the nail bed arteriovenous anastomoses are present, and measurements are not necessarily representative for subcutaneous capillaries. According to the Starling equation, the calculated capillary pressure represents a functional average pressure for the portion of the microvascular bed taking part in fluid exchange. Because of the large surface area and permeability of the venous end of the capillaries and the venules, the anatomic location of the capillary hydrostatic pressure according to this definition may well be in the venules rather than in the capillaries.[8]

In normal pregnancy, the capillary pressure increased on average by 31% between the first and third trimesters. This was observed on both thorax and at the ankle. A rise in capillary hydrostatic pressure can result either from a lowering of the precapillary to postcapillary resistance ratio or an increased venous or arterial blood pressure. It is known that venous blood pressure is unchanged in pregnancy[9, 10] if the measurements are not done in the lower extremities and the woman is not lying on her back. The arterial blood pressure was almost unchanged. This must mean that precapillary to postcapillary resistance ratio is lower in the third trimester of normal pregnancy, probably because of a reduction of precapillary resistance. The increased capillary pressure could be the result of a diminution in the amount of circulating pressor sub-

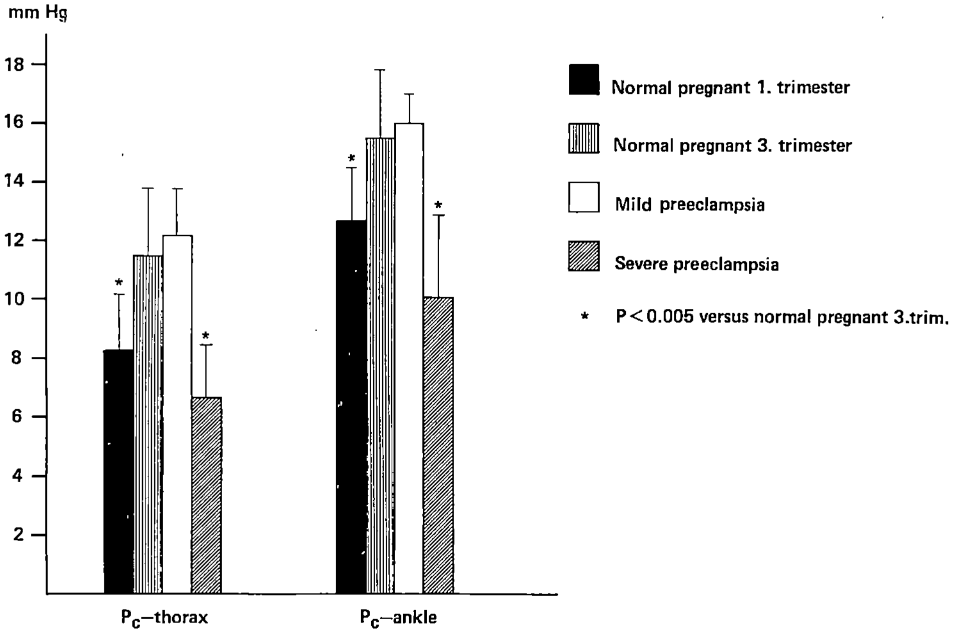

Fig. 1. Calculated capillary hydrostatic pressure *(Pc)* on thorax and at the ankle in normal pregnant women in the first trimester (n = 10) and third trimester (n = 10) and in mild preeclamptic (n = 15) and severe preeclamptic patients (n = 13).

stances, an increase in vasodilator substances, changed sensitivity of the vascular smooth muscle, or a combination of these.

In the group with mild preeclampsia, the average capillary pressure was only slightly increased compared with the third-trimester normotensive group. As shown by Lim and Walters,[11] mild hypertension in pregnancy is associated with a hyperkinetic cardiac function, which is manifested by a faster heart rate, higher cardiac output, and higher total peripheral resistance. These findings indicate that blood pressure regulation is altered at both central and peripheral sites. Obviously, these hemodynamic changes in patients with mild preeclampsia are counteracted by a resetting of precapillary to postcapillary resistance ratio so that capillary hydrostatic pressure is almost unchanged (autoregulation of capillary hydrostatic pressure).

In the severe preeclamptic group, average blood pressure was 174 ± 10/115 ± 6 mm Hg. Despite this, capillary hydrostatic pressure was reduced by 45% on thorax and 39% at the ankle compared with that in the mild preeclamptic group. The low capillary hydrostatic pressure found in severe preeclampsia may be explained by increased vascular resistance. Actually, it is well known that increased vascular resistance, decreased circulating plasma volume, and impaired perfusion to various organs are important features of severe preeclampsia. Severe preeclampsia is also characterized by an exquisite sensitivity of the vasculature for pressor substances.[12] According to Gant and Worley,[13] this leads to a general vasospasm that causes overt hypertension and then decreased intravascular volume. Thus the constriction of the intravascular compartment seems to be the primary event that leads to reduced plasma volume rather than the reverse.[13] Otherwise, one could speculate that the extreme vasoconstriction and low capillary pressure in subcutaneous tissue are secondary responses that promote absorption of fluid into the vascular compartment in an effort to restore the contracted plasma volume in severe preeclampsia.

As stated previously, the calculated capillary hydrostatic pressure is a minimum value, since lymph flow cannot be less than zero. In a steady state (i.e., constant interstitial fluid volume), net capillary filtration (F) must equal lymph flow (L), and calculated capillary hydrostatic pressure is underestimated by lymphatic flow (L) divided by capillary filtration coefficient (CFC) (Equation 1).[8] This has the dimension of pressure. Assuming an unchanged capillary filtration coefficient in preeclampsia compared with normal pregnancy as found by Spetz,[14] the underestimation is proportional to lymph flow. We are unaware of any data that indicates increased lymph flow in preeclampsia. Our patients with severe preeclampsia had larger edema, and their weight gain was more pronounced than those patients with mild preeclampsia. Since most of the retained fluid is located in the interstitial compartment and interstitial colloid osmotic pressure is higher in severe preeclampsia, this implies increased interstitial protein mass. This is probably because of increased capillary permeability to plasma proteins, and therefore σ may be slightly lower in severe preeclampsia than in mild preeclampsia and normal pregnancy.[15] Thus in severe preeclampsia capillary hydrostatic pressure may be even lower than calculated.

The pericapillary fluid distribution is influenced by the transcapillary colloid osmotic gradient (colloid osmotic pressure in plasma − interstitial fluid). In patients with severe preeclampsia this gradient was significantly lower than in patients with mild preeclampsia and in normal pregnant women. A reduced gradient favors transport of fluid from the vascular to the interstitial compartment, and this may explain why many patients with severe preeclampsia have large edema and reduced plasma volume.

In conclusion, in severe preeclampsia capillary hydrostatic pressure in subcutaneous tissue is reduced by about 40% compared with that in normal pregnancy and mild preeclampsia. Whether the low capillary hydrostatic pressure is caused by the severe general vasospasm seen in this condition or is a secondary event remains to be seen.

REFERENCES

1. Guyton AC. Human physiology and mechanisms of disease. Philadelphia: WB Saunders, 1982:228-32.
2. Starling EH. On the absorption of fluids from the connective tissue spaces. J Physiol 1896;19:312.
3. Noddeland H. Colloid osmotic pressure of human subcutaneous interstitial fluid sampled by nylon wicks: evaluation of the method. Scand J Clin Lab Invest 1982;42:123.
4. Øian P, Maltau JM, Noddeland H, Fadnes HO. Oedema-preventing mechanisms in subcutaneous tissue of normal pregnant women. Br J Obstet Gynaecol 1985;92:1113.
5. Fadnes HO, Reed RK, Aukland K. Interstitial fluid pressure in rats measured with a modified wick technique. Microvasc Res 1977;14:27.
6. Aukland K, Johnsen HM. A colloid osmometer for small fluid samples. Acta Physiol Scand 1974;90:485.
7. Pappenheimer JR, Soto-Rivera A. Effective osmotic pressure of the plasma proteins and other quantities associated with the capillary circulation in the hindlimbs of cats and dogs. Am J Physiol 1948;152:471.
8. Aukland K, Nicolaisen G. Interstitial fluid volume: local regulatory mechanisms. Physiol Rev 1981;61:556.
9. McLennan CE. Antecubital and femoral venous pressure in normal and toxemic pregnancy. AM J OBSTET GYNECOL 1943;45:568.
10. Hytten F, Chamberlain G. Clinical physiology in obstetrics. Boston: Blackwell Scientific Publications, 1980:13.
11. Lim YL, Walters WAW. Haemodynamics of mild hypertension in pregnancy. Br J Obstet Gynaecol 1979;86:198.
12. Gant NF, Daley GL, Chand S, Whalley PJ, MacDonald PC. A study of angiotensin II pressor response throughout primigravid pregnancy. J Clin Invest 1973;52:2682.
13. Gant NF, Worley RJ. Hypertension in pregnancy. Norwalk, Connecticut: Appleton-Century-Crofts, 1980:45.
14. Spetz S. Peripheral circulation in pregnancy complicated by toxaemia. Acta Obstet Gynecol Scand 1965;44:243.
15. Øian P, Maltau JM, Noddeland H, Fadnes HO. Transcapillary fluid balance in pre-eclampsia. Br J Obstet Gynaecol 1986;93:235.

Mechanisms for reduced colloid osmotic pressure in preeclampsia

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The determinants of plasma colloid osmotic pressure were studied in 32 patients with preeclampsia and their matched control subjects. Although plasma colloid osmotic pressure was significantly related to preeclampsia, its severity, and proteinuria, it was most highly correlated with an elevated fibronectin level, suggesting that endothelial injury, rather than proteinuria, is the major mechanism of reduced colloid osmotic pressure in preeclampsia. (AM J OBSTET GYNECOL 1987;157:106-8.)

Key words: Plasma colloid osmotic pressure, proteinuria, plasma fibronectin, preeclampsia

Reduced plasma colloid osmotic pressure in preeclampsia has been attributed to increased capillary permeability, presumably because of injury to capillary endothelium of the glomeruli and/or peripheral vasculature.[1,2] However, studies directly linking reduced plasma colloid osmotic pressure to capillary endothelial

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Table I. Plasma colloid osmotic pressure and plasma fibronectin (mean ± SD) levels in control subjects (n = 32), patients with mild preeclampsia (n = 12), and those with severe preeclampsia (n = 20)

	Mild preeclampsia	*Severe preeclampsia*	*Control*	*p*
Colloid osmotic pressure (mm Hg)	18 ± 2	17 ± 2	19 ± 2	0.01
Fibronectin (μg/ml)	444 ± 122	401 ± 102	217 ± 61	0.001

damage in preeclampsia are lacking. Recently, it has become more practical to evaluate capillary endothelial damage by measuring plasma fibronectin levels, which rise with endothelial cell injury or repair.[3, 4] The purpose of this study was to evaluate the interrelationships of plasma colloid osmotic pressure, proteinuria, and plasma fibronectin among normal and preeclamptic pregnancies.

Methods

After approval from the Human Investigation Committee, 64 patients were selected from the outpatient clinic and labor rooms of Hutzel Hospital, Wayne State University. Each of the 32 pregnancies complicated by preeclampsia, 12 mild (blood pressure >140/90 but <160/100 mm Hg) and 20 severe (blood pressure >160/100 mm Hg), were matched for age, parity, gestational age, and labor status with a control patient. Plasma colloid osmotic pressure was measured in triplicate in each patient by a membrane transducer system (Wescor Osmometer 4400, Wescor Inc., Logan, Utah); the instrument was calibrated with a solution of preassayed colloid osmotic pressure, and the mean of three determinations was considered for statistical analysis. Plasma fibronectin measurements were performed by turbidimetric immunoassay (Multistat III centrifugal analyzer), and proteinuria was semiquantitated by dipstick (0 to 4+). Results were analyzed with the multivariate analysis of variance, stepwise multiple regression. A two-tailed $p < 0.05$ was considered significant.

Results

The age and gestational age ranges of the 32 patients with preeclampsia and their control subjects were 19 to 31 years and 23 to 42 weeks, respectively. Age, parity, gestational age, and labor status in the preeclampsia and control groups were comparable as a result of the study design. The means ± SDs of blood pressures were 113 ± 10/68 ± 9 in the control group, 147 ± 8/94 ± 4 in the patients with mild preeclampsia, and 178 ± 15/109 ± 8 in the patients with severe preeclampsia. Plasma colloid osmotic pressure and fibronectin levels were significantly altered in preeclampsia (mild and severe) as compared with control pregnancies, as shown in Table I. The incidence of proteinuria was 0/32 in the control group, 6/12 in patients with mild preeclampsia (four 1+ and two 2+), and 13/20 in patients with severe preeclampsia (one 1+, seven 2+, and five 3+).

Table II. Stepwise multiple regression analysis of factors related to plasma colloid osmotic pressure

	Incremental R^2	*F to enter*	*p*
Fibronectin	0.19	14.5	<0.001
Proteinuria	0.05	4.1	<0.05
Mild preeclampsia (yes/no)	0.02	1.0	NS
Severe preeclampsia (yes/no)	0.01	0.7	NS
Any preeclampsia (yes/no)	0.00	0.0	NS

To further evaluate the genesis of reduced plasma colloid osmotic pressure in preeclampsia, stepwise multiple regression on colloid osmotic pressure was performed (Table II). Plasma colloid osmotic pressure was significantly related to plasma fibronectin, proteinuria, preeclampsia (yes/no), and severe preeclampsia on a univariate basis ($p < 0.01$ in each case). However, in the multivariate setting, plasma fibronectin and proteinuria were more closely linked with plasma colloid osmotic pressure than preeclampsia or its severity.

Comment

The key finding of this study is the close association of reduced plasma colloid osmotic pressure with increased plasma fibronectin level in preeclampsia. The primary cause of this lowered plasma colloid osmotic pressure is generally thought to be significant proteinuria.[2] Our findings suggest that an increased plasma fibronectin level, most reasonably interpreted as representing generalized endothelial damage, and proteinuria are involved in the genesis of reduced plasma colloid osmotic pressure in preeclampsia and thus support the observations of Oian et al.[1]

On the basis of incremental variance, vascular damage appears to be an approximately fourfold more important determinant of plasma colloid osmotic pressure than proteinuria. Even though plasma fibronectin levels in mild and severe preeclampsia were not significantly different, increased levels of proteinuria in severe preeclampsia might be attributed to higher blood pressures in these patients.[1] However, these findings should be interpreted with caution since proteinuria was determined only semiquantitatively in this

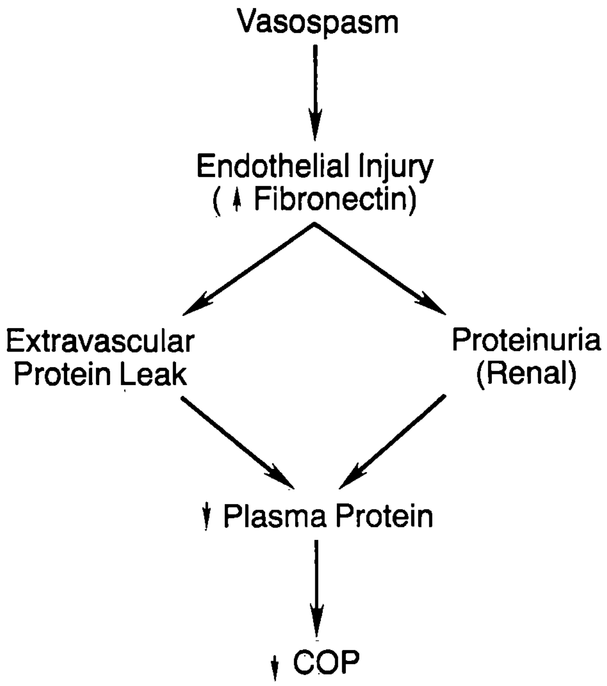

Fig. 1. Possible mechanism of lowered plasma colloid osmotic pressure in pregnancy-induced hypertension.

study and preeclampsia and its severity were used as discrete variables in the multiple regression.

On the basis of these findings, the following mechanism of lowered plasma colloid osmotic pressure in preeclampsia is suggested (Fig. 1). Generalized vasospasm in preeclampsia may be thought of as leading to capillary endothelial injury, elevated plasma fibronectin levels, and increased capillary permeability. Increased capillary permeability along with elevated blood pressure leads to protein loss, both in the interstitium and in urine, which lowers plasma colloid osmotic pressure levels. This model might have important clinical implications. Low plasma colloid osmotic pressure in preeclampsia has been described as a causative factor in the genesis of pulmonary edema. Routine use of intravenous colloid solution in preeclampsia is recommended by some to expand the intravascular volume.[5] Our finding of endothelial damage in preeclampsia suggests that caution should be exercised in giving colloids as they may leak into the interstitium and cause or worsen pulmonary edema.

REFERENCES

1. Oian P, Maltau JM, Noddeland H, Fadnes HO. Transcapillary fluid balance in preeclampsia. Br J Obstet Gynaecol 1986;93:235-9.
2. Chesley L. Hypertensive disorders of pregnancy. New York: Appleton-Century-Crofts, 1978:115.
3. Stubbs TM, Lazarchick J, Horger EO. Plasma fibronectin levels in preeclampsia: a possible biochemical marker for vascular endothelial damage. AM J OBSTET GYNECOL 1984;150:885-7.
4. Haitas B, Barnes AJ, Cederholm-Williams SA, Moore J, Shogry ME, Turner RC. Abnormal endothelial release of fibrinolytic activity and fibronectin in diabetic microangiopathy. Diabetologia 1984;27:493-6.
5. Goodlin RC, Cotton DB, Haesslein HC. Severe edema-proteinuria-hypertension gestosis. AM J OBSTET GYNECOL 1978;132:595.

Abnormalities in platelet antiglobulin tests in preeclamptic mothers and their neonates

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We prospectively studied 40 women with preeclampsia and 26 women with normal pregnancy for the presence of platelet-bound and circulating platelet-bindable immunoglobulin and complement. Although only 12 patients with preeclampsia had a platelet count $<150,000/mm^3$, 36 of 40 demonstrated an abnormal direct antiglobulin test, compared with only three of 26 control subjects ($p < 10^{-8}$). An abnormal indirect test was also detected in 30 of 40 patients with preeclampsia compared with five of 26 healthy pregnant control women ($p = 9.3 \times 10^{-6}$). Abnormal antiglobulin tests persisted for 2 to 6 weeks after delivery. Although each neonate had a platelet count $>200,000/mm^3$ at the time of delivery, 10 of 18 had an abnormal direct antiglobulin test compared with one of 14 control subjects ($p = 0.0049$). The high frequency of abnormal platelet antiglobulin tests in women with preeclampsia and their neonates may indicate an immune cause of certain aspects of the syndrome or may reflect the extent of platelet activation. (Am J Obstet Gynecol 1987;157:109-13.)

Key words: Preeclampsia, platelet antiglobulin test, thrombocytopenia

Preeclampsia is a common obstetric disorder, affecting approximately 7% of primigravid women. More than 10% of patients with preeclampsia develop thrombocytopenia, which may precede other manifestations of the disorder[1] and may persist for many days after delivery.[2] Bleeding caused by severe thrombocytopenia can be the most serious complication in some of these patients. In addition, thrombocytopenia has been observed in up to 25% of neonates born to mothers with preeclampsia.[3] Platelet activation and consumption may also play a direct role in the development of intravascular thromboses that occur in women with severe preeclampsia.[4, 5]

The mechanism by which thrombocytopenia develops in both mothers with preeclampsia and their neonates is unknown. Thrombocytopenia may result from disseminated intravascular coagulation in women who develop eclampsia, but the evidence that thrombin-induced intravascular coagulation causes thrombocytopenia in preeclampsia is far less definitive.[5, 6] Various studies have suggested an immune cause of certain aspects of the preeclampsia syndrome.[7-9] We therefore performed antiglobulin tests on platelets from women with preeclampsia to gain further insight into the mechanism of platelet activation and consumption in this disorder.

Methods

Patient populations. We studied 40 patients with preeclampsia who presented to the Hospital of the University of Pennsylvania between November 1984 and May 1986. The following criteria were used to establish the diagnosis of preeclampsia: (1) A normal blood pressure had been recorded for each patient who had sought prenatal care during the first and second trimesters; (2) at term, each patient had a diastolic blood pressure >90 mm Hg recorded in the left lateral decubitus position on at least two occasions by measurements made at least 6 hours apart; (3) no patient had a history of renal disease; (4) each patient had proteinuria on a catheterized specimen (Albustix); (5) each patient had a serum uric acid concentration >6.0 mg/dl; (6) each patient had a negative test for antinuclear antibodies.

We also studied a control population consisting of 26 pregnant patients matched for age, race, and parity (Table I). The average gestational age was lower in the patients with preeclampsia (Table I). Eight of the pa-

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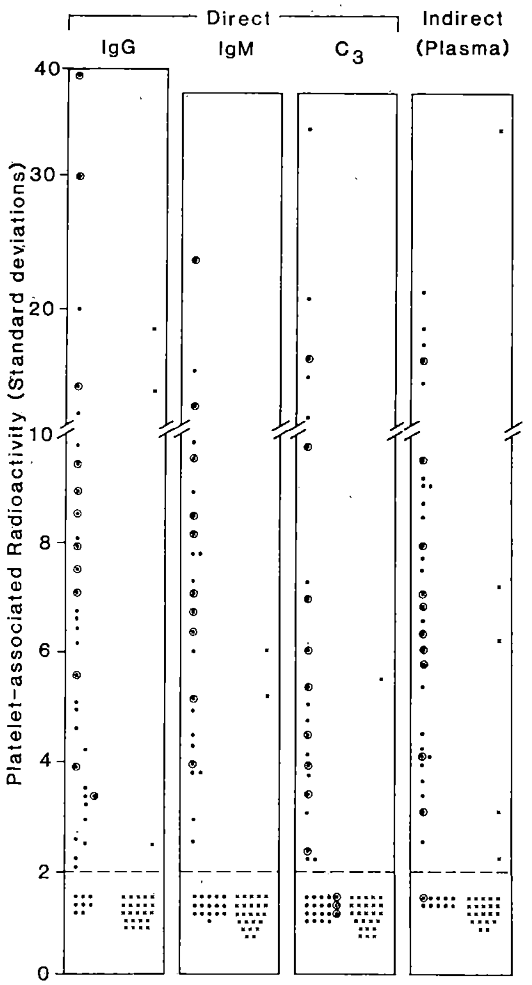

Fig. 1. Maternal platelet antiglobulin tests. Platelet antiglobulin tests were performed on platelets and plasma from women with preeclampsia with normal (●) and decreased (◎) platelet counts and healthy pregnant (x) women. In Figs. 1 and 2, the *dotted line* represents cell-associated radioactivity (mean + 2 SD) in platelets derived from the normal, nonpregnant population.

Table I. Characteristics of patients studied

	Preeclampsia (N = 40)	*Control (N = 26)*
Mean age (yr)	21.3	22.1
Age range (yr)	16-25	18-28
Mean gestation (wk)	35.7	37.5
Gestational range (wk)	27-40	34-41
Primigravid women	36	24
Black/white ratio	29/11	20/6
Thrombocytopenia		
100-150,000/mm^3	6	0
<100,000/mm^3	6	0
No prenatal care	8	0

-tients with preeclampsia had not sought prenatal care, whereas all control patients had been under the care of a physician prior to delivery. None of the control patients had thrombocytopenia, whereas 12 (30%) of the patients with preeclampsia had a platelet count <150,000/mm^3 noted on at least one determination. Six of these patients (15% of the total) had platelet counts <100,000/mm^3. No patient had received magnesium sulfate prior to the time of study. All patients had intact membranes, and none had received corticosteroids to enhance fetal lung maturity.

Platelet antiglobulin tests. Platelet antiglobulin tests were performed by means of a radioimmunoassay within 4 hours after the blood was obtained.[10] Briefly, blood was collected in ethylenediaminetetraacetic acid and the platelets were isolated as described.[10] Platelets, 1 × 10^8, were washed in an ethylenediaminetetraacetic acid–containing modified Tyrode's buffer solution and were incubated with an aliquot of iodine 125–labeled antihuman immunoglobulin G (IgG), immunoglobulin M (IgM), or the third component of complement (C3) for 45 minutes at 37° C. The platelets were washed four additional times in Tyrode's buffer to remove unbound antibody, and the cell-associated radioactivity was determined. The extent of binding of each antiglobulin reagent to platelets is expressed as a percentage of the total radioactivity to which the platelets were exposed according to the formula: Percent binding = platelet-associated counts per minute − test tube–associated counts per minute/total cpm − test tube–associated counts per minute × 100. The mean ± 2 SD from the mean of the percent binding of each reagent to platelets from more than 200 normal donors was determined. Values >2 SD from the mean (IgG = 0.08% ± 0.08%; IgM = 0.16% ± 0.07%; C3 = 0.08 ± 0.08%) were considered abnormal.

In the indirect test, 10^8 platelets from normal donors were incubated with plasma from patients or control subjects for 45 minutes at 37° C. These platelets were washed in Tyrode's buffer and then incubated with ^{125}I-labeled anti-IgG. The cells were then washed four times and platelet-associated radioactivity was determined as in the direct test. Values for plasma-bindable IgG that were more than 2 SD above the mean for normal plasma donors (0.60% ± 0.34%) were considered abnormal. Results for all tests were analyzed by the Fisher exact test for statistical significance.

We also compared the binding to platelets of each labeled antiglobulin reagent with the binding of ^{125}I-labeled normal rabbit IgG not having antibody activity. Platelets from each patient with preeclampsia bound an amount of normal rabbit IgG that was within the range for the binding of rabbit IgG to platelets from normal individuals (0.04% ± 0.04%; mean ± 2 SD).

Results

We performed antiglobulin tests on platelets from women with preeclampsia and from healthy pregnant women. These results were compared with those of antiglobulin tests performed on platelets from a normal, nonpregnant control population. Thirty-six of 40 patients with preeclampsia had one or more abnormal direct antiglobulin tests, compared with four of 26 healthy pregnant women ($p < 10^{-8}$) (Fig. 1). The most frequently observed abnormality was an increase in the level of platelet-associated IgG, which was found in 32 of 40 mothers with preeclampsia compared with only three of 26 control subjects ($p = 3.2 \times 10^{-8}$) (Fig. 1). An abnormal indirect antiglobulin test was seen in 30 of 40 patients with preeclampsia compared with five of 26 healthy pregnant women ($p = 9.3 \times 10^{-6}$) (Fig. 1). Serial studies were performed in two primigravid women. Both had normal antiglobulin tests before and during the second trimester of pregnancy. They developed abnormal platelet antiglobulin test results at 35 and 36 weeks, respectively, coincident with the development of edema and weight gain. They later developed true preeclampsia. There was no correlation between the titer of any of the antiglobulin tests and either the platelet count or any other clinical parameters in the patients with preeclampsia.

We then studied platelets and plasma in umbilical cord blood samples obtained from neonates born to mothers with and without preeclampsia. Ten of 18 neonates born to mothers with preeclampsia had an abnormal direct antiglobulin test result, compared with one of 14 control women ($p = 0.0049$) (Fig. 2). An increased amount of platelet-associated IgG was the abnormality most frequently detected in cord blood of neonates born to mothers with preeclampsia (nine of 18) compared with cord blood of neonates born to healthy mothers (0 of 14) ($p = 0.0017$) (Fig. 2).

Increased amounts of IgM and C3, respectively, were found in the platelets of nine of 15 and five of 15 neonates born to preeclamptic mothers (Fig. 2). Abnormal indirect tests were seen in 11 of 18 neonates born to preeclamptic mothers compared with four of 14 in the control group ($p = 0.07$). In many cases, the mother and neonate differed in the serologic abnormalities detected. Moreover, neither the level of platelet-associated IgG, IgM, or C3 in maternal platelets nor the level of circulating platelet-bindable IgG in maternal plasma correlated with the results of the platelet antiglobulin tests in the neonate. No child in this series had a cord blood platelet count $<200{,}000/mm^3$. There was no correlation between the magnitude of any of the results of the antiglobulin tests and the platelet counts of the neonates.

We performed serial antiglobulin tests on platelets from six women post partum (Fig. 3). Both the direct and indirect antiglobulin tests returned to normal in each case by 6 weeks after delivery. The exact time point at which tests reverted to normal was not determined. One patient experienced a severe exacerbation of symptoms in the postpartum period, and a concurrent rise in the level of both platelet-bound IgG and circulating platelet-bindable IgG was detected.

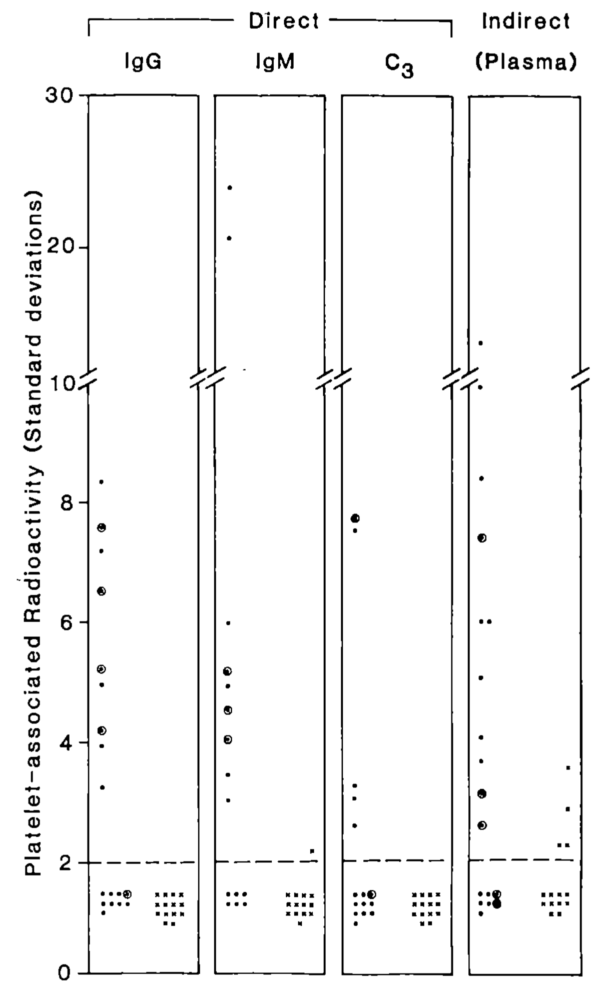

Fig. 2. Platelet antiglobulin tests on cord blood. Platelet antiglobulin tests were performed with platelets or plasma obtained from neonates born to healthy women (x) or to women with preeclampsia who had normal (●) or decreased (⊙) platelet counts.

Comment

Thrombocytopenia develops in approximately 10% of women with preeclampsia and in a higher percentage of women with progression to eclampsia. Occasionally, patients may present with thrombocytopenia as the most serious clinical finding.[1] The platelet count may fall to levels $<20{,}000/mm^3$, and thrombocytopenia may persist after the other manifestations of the syndrome have subsided. Thrombocytopenia also develops in some neonates born to mothers with preeclampsia.[3]

The responsible mechanism by which thrombocytopenia develops in preeclampsia is uncertain. In preeclampsia, a progressive fall in platelet number has been reported to develop as early as 28 weeks of gestation. Platelet survival appears to be reduced even in the absence of overt thrombocytopenia. Increased

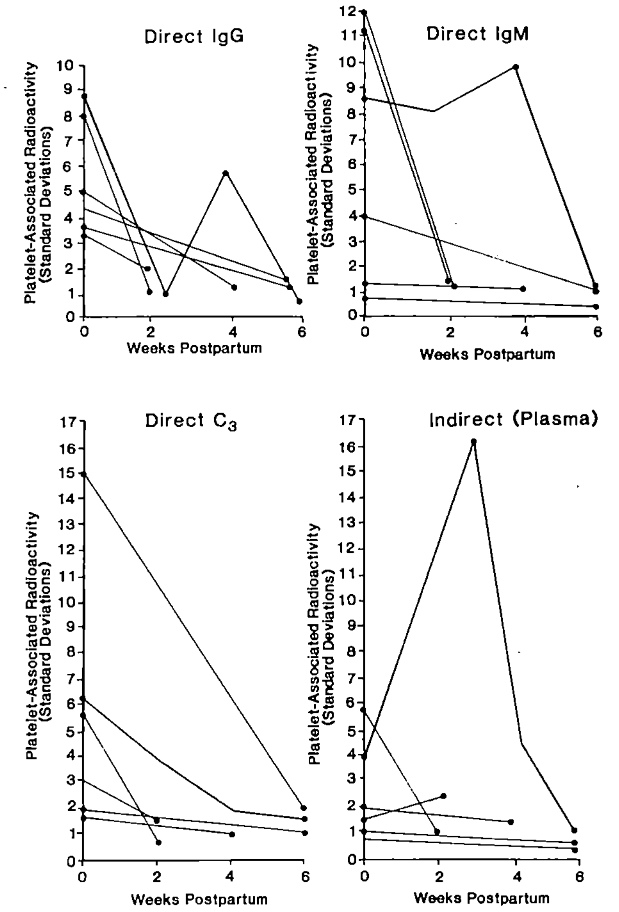

Fig. 3. Serial platelet antiglobulin tests in preeclampsia. The zero time point represents the immediate postdelivery period.

levels of β-thromboglobulin and platelet factor 4, a reduced platelet content of 5-hydroxytryptamine, and impaired production of thromboxane B_2 have all been reported. These observations suggest that platelets may be activated in vivo in these patients.

Several mechanisms have been proposed to explain both the reduction of platelet number and the evidence for platelet activation. These include the generation of thrombin, the presence of circulating immune complexes, and the effect of vascular disruption. Although patients with eclampsia may have evidence of disseminated intravascular coagulation, the role of thrombin generation in preeclampsia is less certain. Both a mild increase in the serum levels of fibrin degradation products[5] and a fall in the plasma level of antithrombin III have been noted by a number of investigators. However, sequential studies of markers of fibrin degradation, platelet activation, and platelet consumption have been interpreted as indicating that the extent of thrombin generation cannot account for the development of thrombocytopenia in most patients.[5] The use of heparin in women with preeclampsia does not cause the platelet count to rise, but low doses of aspirin alone or aspirin in combination with dipyridamole have been reported to prevent and/or reverse the development of thrombocytopenia in some patients.[11]

Immune platelet injury could play a role in the development of thrombocytopenia in some patients with preeclampsia. In agreement with others,[12-14] we found increased levels of platelet-associated IgG and/or IgM and/or C3 in most patients with preeclampsia as compared with a much lower proportion of platelets from healthy pregnant control subjects. Our study was similar to a recent one in that we found increased levels of platelet-associated immunoglobulin and complement in platelets obtained from preeclamptic cord blood samples.[12] Plasma from patients with preeclampsia contained increased amounts of platelet-bindable IgG compared with plasma from healthy pregnant women of similar parity and length of gestation. Abnormal platelet antiglobulin tests return to normal by 6 weeks after delivery.

The mechanism by which increased platelet-associated immunoglobulin and complement develop is unclear. At present, we do not know whether the platelet-bound immunoglobulin results from the deposition of autoreactive antibody or immune complexes. Increased levels of immune complexes have been noted in the plasma and tissues of some patients with preeclampsia when compared with those of healthy pregnant control women.[7-9, 12-15] However, there has been no identification of the antigens involved, nor has the relevance of these complexes to thrombotic vascular disease been established. A preliminary study indicates that antigens released from placental tissue could be involved in the generation of these complexes. Alternatively, activation of platelets at sites of microvascular injury could lead to: (1) externalization of IgG and other proteins found within the α-granules of the platelet,[16] (2) increased expression of platelet Fc receptors, (3) exposure of cryptic antigens to which low levels of naturally occurring autoreactive antibodies may exist, or (4) exposure of determinants that permit adsorption of normal proteins from the plasma.

Neonates born to mothers with preeclampsia may develop thrombocytopenia, neutropenia, edema, proteinuria, and abnormal liver function tests.[3] These findings indicate either that a causal factor, present in the plasma of women with preeclampsia, is capable of crossing the normal placenta or that the preeclamptic process damages the placenta, causing a breakdown in the normal process of selective protein transfer. There were increased levels of IgG in the platelets of neonates born to preeclamptic mothers, and these platelets also bound increased amounts of anti-IgM. Damage to placental vessels by the preeclamptic process could conceivably allow transplacental passage of maternal IgM. Alternatively, transplacental passage of maternal IgG may initiate IgM production by the fetus. Another possibility is that a plasma factor, perhaps produced by the placental syncytiotrophoblast, may be released into the fetal and maternal circulations, initiating an immune response.

Our findings in the healthy pregnant population differ from those of a recent report in which a high frequency of abnormal antiglobulin tests was found in this group of patients.[17] Only a small number of the healthy women whom we studied had abnormal antiglobulin tests at term or in the peripartum period, and the magnitude of these changes was small compared with that of most patients with preeclampsia or immune thrombocytopenic purpura.[18] The reasons for the differences in the findings of these two studies are not evident. The results of our investigation, however, do indicate that platelet antiglobulin tests cannot be used to distinguish preeclampsia from immune thrombocytopenic purpura in those patients who present with thrombocytopenia alone.

The results of the present study show that the platelet surface is altered in women with preeclampsia and their neonates, even in the absence of bleeding. The molecular composition and immunologic specificity of these platelet-bound proteins are unknown, and their relative importance to the development of either thrombocytopenia or thrombosis requires further investigation.

REFERENCES

1. Goldenberg RL, Huddleston JF, Davis RO, Harris BA. Toxemia of pregnancy masquerading as idiopathic thrombocytopenic purpura. Obstet Gynecol 1983;62 (suppl):32.
2. Schwartz ML, Brenner WE. Pregnancy-induced hypertension presenting with life-threatening thrombocytopenia. AM J OBSTET GYNECOL 1983;146:756.
3. Brazy JE, Grimm RN, Little VA. Neonatal manifestations of severe maternal hypertension occurring before the thirty-sixth week of pregnancy. J Pediatr 1982;100:265.
4. Whigham KAE, Howie PW, Drummond AH, Prentice CRM. Abnormal platelet function in preeclampsia. Br J Obstet Gynaecol 1978;85:28.
5. Norok Z, Weitz J, Owen J, Auerbach M, Nossel HL. Fibrinogen proteolysis and platelet alpha-granule release in preeclampsia/eclampsia. Blood 1984;63:525.
6. Gibson B, Hunter D, Neame PB, et al. Thrombocytopenia in preeclampsia and eclampsia. Semin Thromb Hemost 1982;8:234.
7. Kitzmiller JL, Benirschke K. Immunofluorescent study of placental blood vessels in preeclampsia of pregnancy. AM J OBSTET GYNECOL 1973;115:248.
8. Petrucco OM, Thomson NM, Lawrence JR, Weldon MW. Immunofluorescent studies in renal biopsies in preeclampsia. Br Med J 1974;1:473.
9. Houwert-de Jong MH, te Velde ER, Nefkens MJJ, Schuurman HJ. Immune complexes in skin of patients with preeclamptic toxemia. Lancet 1982;1:387.
10. Cines DB, Tomaski A, Wilson S, Schreiber AD. Platelet antibodies of the IgM class in immune thrombocytopenic purpura. J Clin Invest 1985;75:1183.
11. Wallenburg HCS, Makovitz JW, Dekker GA, Rotmans P. Low-dose aspirin prevents pregnancy-induced hypertension and preeclampsia in angiotensin-sensitive primigravidae. Lancet 1986;1:1.
12. Rote NS, Harrison M, Lau RJ, Scott JR. Thrombocytopenia in pregnancy-induced hypertension. Ann NY Acad Sci 1986;425:373.
13. Kelton JG, Powers PJ, Carter CJ. A prospective study of the usefulness of the measurement of platelet-associated IgG for the diagnosis of idiopathic thrombocytopenic purpura. Blood 1982;60:1050.
14. Massobrio M, Benedetto C, Bertini E, Tetta C, Camussi G. Immune complexes in preeclampsia and normal pregnancy. AM J OBSTET GYNECOL 1985;152:578.
15. Medcalf RL, Kuhn RJP, Iwanov V, Mathews JD, Moulds RFW. Vasoactivity generated from platelets by immune complexes in preeclampsia. Clin Exp Pharmacol Physiol 1983;10:369.
16. George JN, Saucerman S, Levine SP, Knieriem LK, Bainton DF. Immunoglobulin G is a platelet alpha granule-secreted protein. J Clin Invest 1985;76:2020.
17. Hart D, Dunetz C, Nardi M, et al. An epidemic of maternal thrombocytopenia associated with elevated antiplatelet antibody. AM J OBSTET GYNECOL 1986;154:878.
18. Cines DB, Dusak B, Tomaski A, Mennuti M, Schreiber AD. Immune thrombocytopenia in pregnancy. N Engl J Med 1982;306:826.

Genetic implications of idiopathic hydramnios

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The antenatal diagnosis of hydramnios requires a careful search for associated underlying maternal or fetal conditions. Even in pregnancies associated with idiopathic hydramnios in which no underlying condition can be identified, a high perinatal mortality rate exists. Although trisomy 18 has been seen in pregnancies with hydramnios and growth retardation, the association with specific chromosomal disorders and idiopathic hydramnios has not been well defined. A review of pregnancies complicated by hydramnios was undertaken; 99 cases with complete pregnancy and delivery information were identified. Fifty-nine pregnancies were complicated by idiopathic hydramnios, and except for the hydramnios, sonographic evaluation was normal. Delivery information revealed one infant with trisomy 18 and eight infants with structural anomalies not appreciated antenatally. Of the undiagnosed malformations, 25% are frequently associated with trisomy 21. We recommend fetal chromosomal analysis as an adjunct to the evaluation of pregnancies complicated by idiopathic hydramnios. (AM J OBSTET GYNECOL 1987;157:114-7.)

Key words: Hydramnios, trisomy 18, fetal anomalies

Hydramnios is a relatively common obstetric problem and has been associated with many maternal and fetal complications. The diagnosis of hydramnios requires careful evaluation of the pregnancy for possible maternal diabetes mellitus, multiple gestation, fetal hydrops, or fetal congenital anomalies. Some fetal malformations that can be seen sonographically have been shown to be associated with chromosomal aberrations.[1-4] The association of specific chromosomal disorders and idiopathic hydramnios, however, has not been well defined, although the association of trisomy 18 and hydramnios has been described[5-7]; growth retardation may also be present.[5] The benefit of pursuing the fetal karyotype in a normal pregnancy except for hydramnios has been questioned.

The present study was undertaken in an attempt to establish the role of evaluating the fetal karyotype in pregnancies complicated by idiopathic hydramnios.

Material and methods

A review of cases of hydramnios complicating pregnancies between 1979 and 1986 at The George Washington University Medical Center, Washington, D. C., was undertaken. A total of 158 cases were identified retrospectively by review of medical records of patients referred to or receiving primary care at our institution.

Because only cases with complete pregnancy and delivery information, including maternal glucose screening, have been included, many patients referred to the Departments of Obstetrics and Gynecology, Medical Genetics, or Radiology for consultation during their pregnancies were omitted from this review. Cases of hydramnios diagnosed solely on a clinical basis without sonographic confirmation were excluded. In each case, the diagnosis of hydramnios was made sonographically in the second or third trimesters by subjective observation of an experienced sonologist, and the fetal trunk was found to be freely floating in amniotic fluid.

Results

There were 99 cases of pregnancy complicated by hydramnios for which complete pregnancy and delivery information was available. The 13 cases diagnosed on a clinical basis alone were excluded.

Nineteen cases were found to be associated with maternal diabetes mellitus (19.2%), nine with multiple gestations (9.1%), and 12 with associated fetal anomalies identified sonographically (12.1%), including seven cases of fetal hydrops, one of which was also associated with maternal diabetes mellitus (Table I). Two infants were found to have trisomy 21, and both were found to have duodenal atresia: in one infant, the anomaly was diagnosed antenatally, and in the other, the affected infant was one of a set of twins and the anomaly was not appreciated antenatally. Advanced maternal age was not a risk factor in either pregnancy.

In the remaining 59 cases (59.6%), no underlying fetal or maternal condition could be identified antenatally, and except for the hydramnios, the results of sonographic examination were normal. Delivery information in these patients revealed that one infant had trisomy 18 and seven others had structural anomalies

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Table I. Fetal anomalies associated with non-idiopathic hydramnios

Associated condition	*No. of patients*	*Karyotype*
Maternal DM known antenatally	Total 19	
TEF	1	
Hypomelanosis of Ito	1	
Bilateral recurvation of knees	1	46,XY
Common AV canal (stillborn, 30 wk)	1	46,XX
True knot in umbilical cord	1	
Sonographic abnormalities	Total 12	
Hydrops	7	
Nonimmune (unable to resuscitate)	2	46,XX; 46,XY
Fetal ascites, edema (no anomalies)	3	
Omphalocele, herniated liver (associated maternal DM)	1	46,XY
Duodenal atresia	1	47,XY + 21
Fetal anomalies detected antenatally	5	
Heart block	1	
Duodenal atresia	2	46,XX; 46,XX
Cystic hygroma, hydrocephalus, ascites, calyceal dilatation	1	46,XY
Diaphragmatic hernia	1	
Multiple gestations	9 sets	
Twins	8 sets	
1 twin with gastrointestinal atresia detected antenatally	1	46,XX
1 twin with duodenal atresia detected antenatally	1	47,XX + 21
Twin-to-twin transfusion	2	
Triplets	1 set	
1 fetus with acardiac anomaly		46,XX

TEF = Tracheoesophageal fistula; DM = diabetes mellitus; AV = atrioventricular.

of the cardiovascular, respiratory, and/or gastrointestinal systems, and one additional infant had a short-limbed skeletal dysplasia (Table II). In two of these cases, only level I sonograms were used, including those pregnancies that resulted in one infant with duodenal atresia and the infant with trisomy 18, in which the maternal age was 24. Therefore structural anomalies were present and not detected antenatally in eight of 59 fetuses (13.6%).

Only 16 of the 99 patients with pregnancies complicated by hydramnios underwent genetic amniocentesis as part of the prenatal evaluation: Five were performed because of advanced maternal age, four because of sonographically detected fetal anomalies, including one twin pregnancy, and six to evaluate more thoroughly the hydramnios, including one triplet pregnancy. One patient underwent chorionic villus sampling because of advanced maternal age. All karyotypes were normal.

Comment

Hydramnios is estimated to occur in 0.26% to 0.7% of pregnancies,[8, 9] and its diagnosis had largely been made clinically until the development of ultrasonography. The ability to accurately quantitate the amount of amniotic fluid sonographically has been problematic; however, it has been stated that simple observation by an experienced sonographer can determine the relative amount of fluid in assessing hydramnios.[10]

The finding of hydramnios in any pregnancy requires a careful search for any associated underlying condition: maternal diabetes mellitus, multiple gestations, fetal anomalies involving the central nervous system, gastrointestinal tract, respiratory or cardiovascular systems, fetal hydrops, or placental anomalies. As many as 20% of cases of hydramnios have been found to be associated with fetal anomalies.[8, 11] Some fetal malformations may be seen in conjunction with specific genetic disorders.[1-3] A high associated perinatal mortality rate exists. In as many as 34% of pregnancies complicated by hydramnios, no apparent underlying maternal or fetal disorder can be identified antenatally[8]; however, the perinatal mortality rate is still increased. The present study retrospectively examined 59 pregnancies complicated by idiopathic hydramnios in an attempt to assess the role of pursuing the fetal karyotype in further evaluation of these pregnancies.

Pathophysiologically, excess amniotic fluid may result from conditions that compromise the ability of the fetus to swallow, including anomalies of the central nervous system such as anencephaly or meningomyelocele, obstruction of the fetal gastrointestinal tract in cases of duodenal atresia or abdominal wall defects, and intrathoracic anomalies that may exert excess pressure on the upper gastrointestinal tract, such as diaphragmatic hernias or intrathoracic cysts.[11, 12] It may also be seen in cases of fetal hydrops and associated cardiac failure,

Table II. Neonatal outcomes in pregnancies with idiopathic hydramnios and antenatal sonographic evaluation

Neonatal outcome	*Sonographic evaluation*
47,XX + 18	19—level I scan only
TEF	30
TEF	33½
TEF	11; 33½
TEF and esophageal atresia	30; 34
Duodenal atresia	35—level I scan only
Cardiac defect (? tetralogy)	16; 20½; 26
Short-limbed skeletal dysplasia	9; 11; 25½

TEF = Tracheoesophageal fistula.

as well as in multiple gestations. Hydramnios as part of the complications seen in pregnancies of diabetic women is not fully understood, although it is well known that the frequency of congenital malformations is increased in the presence of maternal diabetes mellitus. Transient hydramnios has also been described.[11] Several authors have described pregnancies complicated by hydramnios that were found to involve fetuses with trisomy 18,[5-7] and intrauterine growth retardation has also been seen.[5]

In our study of 59 patients, the antenatal finding of sonographically detectable hydramnios, which was thought to represent idiopathic hydramnios, was in fact associated with one trisomic infant and eight structural anomalies undetected before delivery (Table II). These data imply a false-negative rate of 13.6% in antenatal detection of these anomalies. It must be remembered, however, that only level I ultrasonography was used in two of these patients and that only a single ultrasound examination was performed in four of these pregnancies.

An inability to recognize a fetal anomaly sonographically may be from several factors: (1) inherent difficulties in performing obstetric sonography, including the ability to visualize structures based on intrinsic differences in contrast, maternal body habitus, and fetal movement or position[13, 14]; (2) at the time of sonographic examination, the particular anomaly may not be at a point in development in which it may be recognized[15]; (3) a single malformation may be associated with other anomalies that are not detectable at the time of sonographic evaluation[15]; (4) the anomaly may be difficult to evaluate because of the intrinsic nature of the organ involved, such as the collapsible nature of the esophagus[15] or because the malformation is small or subtle; (5) failure to perform a thorough level II sonogram or to repeat the sonographic evaluation serially; and (6) relative experience, competency, or interest of the sonographer or sonologist.[6, 11, 13] These factors can be applied to each infant in whom malformations are not appreciated antenatally. Compounding this important area of prenatal diagnosis is the fact that abnormal fetuses may indeed have completely normal sonographic examinations, as has been demonstrated with trisomic fetuses.[6]

Two of the undiagnosed malformations in our series (25%) involved esophageal or duodenal atresia, anomalies that may be difficult to diagnose sonographically because the fetal stomach may or may not be visible, which depends on the presence of a communication between the trachea and esophagus proximal to the obstruction.[11, 15] The importance of this finding is the frequent occurrence of this anomaly in infants with trisomy 21.

Based on these data, we recommend the use of fetal chromosomal analysis as an adjunct to the evaluation of pregnancies complicated by hydramnios in which other conditions cannot be elucidated. Once the karyotype is known, the counseling and management plan can be refined. Fetal cells for chromosome analysis may be obtained by amniocentesis or percutaneous umbilical blood sampling.[16] Amniocentesis is the standard technique with an established record of safety and accuracy. In cases of hydramnios, amniocentesis is particularly easy to perform. Percutaneous umbilical blood sampling is a new and more invasive method, which requires highly skilled persons and advanced technology and may have higher morbidity than amniocentesis. The relative difficulty of performing percutaneous umbilical blood sampling versus amniocentesis in hydramnios is unknown. The advantage of percutaneous umbilical blood sampling appears to be the rapidity with which results can be obtained from cord blood compared with cultured amniocytes: several days versus 2 to 3 weeks.

If the karyotype is abnormal and associated with a hopeless, fatal prognosis for the fetus (e.g., trisomy 18), counseling should include preparation of the family for the child's death, whether as a fetus or neonate. Recognition of the dismal prognosis may lead to induction of labor and avoidance of cesarean section despite ominous fetal heart rate patterns, which is often seen with trisomy 18. Nursing staff members can be maximally supportive in knowing the prognosis in advance.

If the karyotype is normal, such reassuring information can lead to aggressive antepartum monitoring and intrapartum planning to optimize fetal salvage. These plans might include delivery at a tertiary care center and coordination with pediatric subspecialties for possible surgical intervention of the newborn infant.

We recognize that a genetic diagnosis is not always definitive. Other genetic disorders not associated with an obviously dismal prognosis may become apparent, such as mosaics, translocations, or sex chromosome abnormalities. We believe that responsible explanations and representations of expected neonatal prognoses can only be proposed when as much information as possible about a pregnancy is known. In caring for patients with pregnancies complicated by idiopathic hydramnios, important information can be obtained through knowledge of the fetal karyotype.

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REFERENCES

1. Platt LD, DeVore GR, Lopez E, et al. Role of amniocentesis in ultrasound-detected fetal malformations. Obstet Gynecol 1986;68:153.
2. Marchese CA, Carozzi F, Mosso R, et al. Fetal karyotype in malformations detected by ultrasound [Abstract 661]. Am J Hum Genet 1985S;37:A223.
3. Morris K, Hesser J, Michalski K, et al. High chromosome abnormality rate from obstetrically indicated late amniocentesis [Abstract 663]. Am J Hum Genet 1985S;37:A223.
4. Nicolaides KH, Rodeck CH, Gosden CM. Rapid karyotyping in non-lethal fetal malformations. Lancet 1986; 1:283.
5. Schneider AS, Mennuti MT, Zackai EH. High cesarean section rate in trisomy 18 births: a potential indication for late prenatal diagnosis. Am J Obstet Gynecol 1981; 140:367.
6. DeVore GR, Hobbins JC. Ultrasound diagnosis of congenital birth defects. In: Depp R, Eschenbach DA, Sciarra JJ, eds. Gynecology and obstetrics. Vol 3. New York: Harper & Row, 1986:27, 35, 40.
7. Sabbagha RE, Sheikh Z, Tamura RK, et al. Predictive value, sensitivity, and specificity of ultrasonic targeted imaging for fetal anomalies in gravid women at high risk for birth defects. Am J Obstet Gynecol 1985;152:822.
8. Queenan JT, Gadow EC. Polyhydramnios: chronic versus acute. Am J Obstet Gynecol 1970;108:349.
9. Jacoby HE, Charles D. Clinical conditions associated with hydramnios. Am J Obstet Gynecol 1966;94:910.
10. Zamah NM, Gillieson MS, Walters JH, et al. Sonographic detection of polyhydramnios: a 5-year experience. Am J Obstet Gynecol 1982;143:523.
11. Hobbins JC, Grannum PA, Berkowitz RL, et al. Ultrasound in the diagnosis of congenital anomalies. Am J Obstet Gynecol 1979;134:331.
12. Quinlan RW, Cruz AC, Martin M. Hydramnios: ultrasound diagnosis and its impact on perinatal management and pregnancy outcome. Am J Obstet Gynecol 1983; 145:306.
13. Hattan RA, Rees GK, Johnson ML. Normal fetal anatomy. Radiol Clin North Am 1982;20:271.
14. Filly RA, Golbus MS. Ultrasonography of the normal and pathologic fetal skeleton. Radiol Clin North Am 1982; 20:310.
15. Knochel JQ, Lee TG, Melendez MG, et al. Fetal anomalies involving the thorax and abdomen. Radiol Clin North Am 1982;20:297.
16. Hobbins JC, Grannum PA, Romero R, Reece EA, Mahoney MJ. Percutaneous umbilical blood sampling. Am J Obstet Gynecol 1985;152:1.

Circulating neurohypophyseal hormones in anencephalic infants

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Human anencephalic infants have always been considered not to have circulating levels of oxytocin or vasopressin. However, this article shows that amniotic oxytocin levels in anencephalic infants without hydramnios fall within the control range. In addition, low levels of both oxytocin and vasopressin are present in the umbilical circulation. These peptides are probably derived from fetal sources other than the fetal brain, for example, the fetal adrenal cortex. (Am J Obstet Gynecol 1987;157:117-9.)

Key words: Oxytocin, vasopressin, anencephalic infants

The fetal brain is hypothesized to influence labor, fetal homeostasis, adaptation of the fetus to stress, and preparation for extrauterine functions by the neuropeptides oxytocin and vasopressin which circulate in blood and amniotic fluid of normal human fetuses.

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Table I. Amniotic oxytocin levels in normal human pregnancies and in some anencephalic infants with and without hydramnios

Infants	n	Gestation length (range, wk)	Amniotic oxytocin (pg/ml)		Samples below detection level (%)	Significance
			Mean ± SEM	Range		
Anencephalic, with hydramnios	7	31-40	<7 ± 4*	<0.25-33	71	p = 0.03
Anencephalic, without hydramnios	10	22-44	22 ± 8	<3-71	30	p = 0.006
Control†	38	36-42½	21 ± 3	<2-88	3	NS

n = Number of cases determined; < = below detection level.

*For calculations the detection levels were used if the values were below this level. Differences were calculated with the Mann-Whitney U test (two-tailed, corrected for ties).

†Control subjects comprise a group of patients in normal labor.

Table II. Oxytocin and vasopressin levels* in human umbilical cord blood in normal human pregnancies after spontaneous labor and in some anencephalic infants

	Oxytocin		Arginine vasopressin	
	Umbilical artery	Umbilical vein	Umbilical artery	Umbilical vein
Spontaneous labor†	40 (65 ± 18)	17 (20 ± 3)	392 (420 ± 78)	135 (147 ± 33)
Anencephalic infant 1	14.7	<10.9	<13.5	<14.1
Anencephalic infant 2	<27.3	30.1	3.9	7.4
Anencephalic infant 3	<15.0	<13.6	3.1	4.2

< = Below detection level of the assay.

*Values are presented in median (mean ± SEM) levels in picograms per milliliter.

†Samples were obtained from 13 patients after normal labor.

During labor increased levels of oxytocin (umbilical circulation and amniotic fluid) and arginine vasopressin (umbilical circulation) have been reported. Anencephalic infants have no neurohypophysis that contains these peptides[1] and have often been studied to obtain clinical support for experimental data concerning the effect of a lack of fetal oxytocin and arginine vasopressin. However, recent observations raised serious doubts as to the absence of fetal neuropeptides in anencephalic infants. We have reported on the presence of normal concentrations of oxytocin in freshly obtained amniotic fluid of eight anencephalic infants without any detectable arginine vasopressin.[2] In this article we offer an extension of our earlier data. In addition, for the first time we make mention of detectable amounts of oxytocin and arginine vasopressin in the umbilical artery and vein of three live-born anencephalic infants, as opposed to the few anencephalic infants reported on in the literature.

The material comprises a total of 17 anencephalic infants, from whom we were able to collect amniotic fluid that had not been contaminated with blood or meconium. The material was subdivided into two groups on the basis of the presence or absence of clinically diagnosed hydramnios. The results are summarized in Table I. In the absence of hydramnios, amniotic oxytocin concentrations were similar to those found in uncomplicated pregnancies. In anencephalic infants with hydramnios, oxytocin often fell below the detection level. On no occasion was arginine vasopressin found in amniotic fluid of anencephalic infants. However, this was also the case in 75% of our samples obtained in normal pregnancies (data not shown).

In three live-born anencephalic infants after intravenous administration of sulprostone to induce labor at 33 to 35 weeks of gestation, detectable amounts of oxytocin and arginine vasopressin were demonstrated in the separate umbilical vessels. These levels were usually lower than those in the circulation of children born after normal vaginal deliveries (Table II). Oxytocin could also be demonstrated in a fourth anencephalic infant, from whom we could only obtain umbilical vein blood (11.5 pg/ml). Earlier we had reported the presence of oxytocin in mixed cord blood of three anencephalic infants.[2]

The present data show that neurohypophyseal peptides circulate in human anencephalic infants. Inevitably they must originate from alternative fetal sources outside the fetal brain, since a passage of oxytocin and arginine vasopressin from the mother to the fetus is unlikely.[3] Because oxytocin was localized in the fetal zone of the adrenal cortex and arginine vasopressin in the definitive zone of the adrenal cortex in both normal control subjects and anencephalic neonates,[3] this organ is a putative source for these circulating fetal neuropeptides. For studies concerning, for example, passage of arginine vasopressin or oxytocin and their possible role in labor and fetal homeostasis, the anencephalic infant can no longer be considered to be a child with no circulating arginine vasopressin or oxytocin.

REFERENCES

1. Visser M, Swaab DF. Life span changes in the presence of alpha-melanocyte-stimulating hormone-containing cells in the human pituitary. J Dev Physiol 1979;1:161.
2. Swaab DF, Oosterbaan HP. Exclusion of the fetal brain as the main source of rat and human amniotic fluid oxytocin. Br J Obstet Gynaecol 1983;90:1160.
3. Ravid R, Oosterbaan HP, Hansen BL, Swaab DF. Localization of oxytocin, vasopressin and parts of precursor in the human neonatal adrenal. Histochemistry 1986; 84:401.

Fetal pulmonary hypoplasia with hydrothorax

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Described is a case of fetal congenital malformation with hydrothorax, for which left pulmonary hypoplasia was initially presumed by ultrasonography and for which hypoplasia was diagnosed postnatally only in the left lower pulmonary lobe by a pulmonary technetium Tc-99m scintigram. The pathogenesis of this disease is discussed. (Am J Obstet Gynecol 1987;157:119-20.)

Key words: Fetal pulmonary hypoplasia, fetal hydrothorax, ultrasound, pathogenesis

Fetal pulmonary hypoplasia, especially lobar hypoplasia, with hydrothorax is an extremely rare intrauterine complication.[1] We report here an interesting case that we experienced recently.

Case report

A 29-year-old woman, gravida 2, para 0, without any recorded medical history presented with a complaint of abdominal fullness at 28 weeks' gestation. Hydramnios and threatened premature delivery were diagnosed, and she was admitted to the hospital.

Ultrasound examination at the twenty-eighth week demonstrated moderate hydramnios, with the lungs and the heart of the fetus compressed by a large amount of fetal intrathoracic fluid (Fig. 1). The fluid was retained only in the left side of the thorax. Although the fetal heart was shifted to the right side, cardiac activity was normal and the four chambers were clearly recognized. The biparietal diameter was 7.6 cm, large but normal for the fetal age. The other organs were normal.

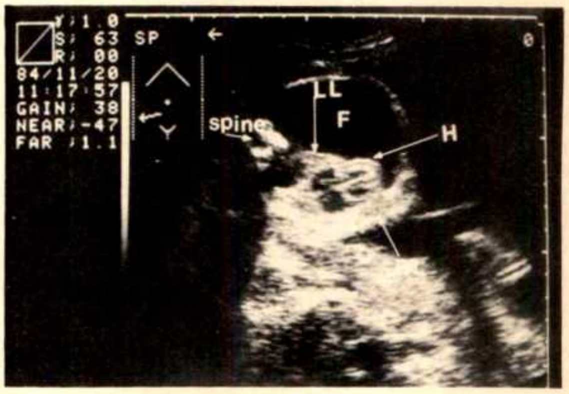

Fig. 1. Oblique scan through fetal thorax showing the left lung *(LL)*, the right lung *(RL)*, and the heart *(H)* compressed by a large amount of intrathoracic fluid *(F)*.

Amniocentesis and fetal thoracentesis were performed under ultrasound guidance at the thirtieth week. Fetal monitoring (nonstress test and cardiotocogram) before and after the puncture showed no abnormal patterns. The amounts of aspirated amniotic and fetal intrathoracic fluid were 200 and 25 ml, respectively; the former was clear and watery and the latter was clear yellow. The aspirated fluids were cytologically negative for malignant cells and also for bacterial growth.

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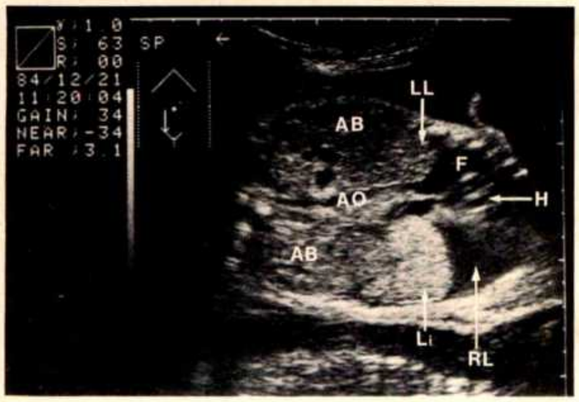

Fig. 2. Longitudinal scan through fetal body. *H*: Heart; *F*: intrathoracic fluid; *LL*: left lung; *RL*: right lung; *Li*: liver; *AO*: aorta; and *AB*: abdomen.

Ultrasonographic examination at the thirty-first week revealed a trend to slight improvement of hydramnios, a small left lung with a large amount of intrathoracic fluid, and a normal-sized right lung (Fig. 2). The left lung was interpreted, from these findings, to be hypoplastic.

In the amniography performed with 40 ml of 60% Urografin after amniocentesis at the thirty-second week, the gastrointestinal tract was not visualized. The lecithin value and the lecithin/sphingomyelin ratio in the amniotic fluid were 85.0% and 5.67, within normal ranges.

Premature rupture of the membranes occurred at the thirty-third week; labor began spontaneously, and a premature male infant weighting 2060 gm was delivered, with Apgar scores of 8 and 9 at 1 and 5 minutes. There was an outflux of a large amount of amniotic fluid. The placenta and umbilical cord were normal.

The infant immediately breathed and cried, but at 10 minutes, respiratory distress prompted immediate intubation and needle thoracentesis. Straw-colored fluid amounting to 120 ml was aspirated from the left thoracic cavity. On transfer to the neonatal intensive care unit, a left chest tube was inserted. After this management, the intrathoracic fluid disappeared spontaneously on the fifth day of life. Hypoplasia only in the left lower pulmonary lobe was confirmed on the twenty-first day by a pulmonary technetium Tc-99m scintigram. The infant subsequently has continued to do well.

Comment

Fetal pulmonary hypoplasia may be either congenital or acquired, and it is considered that compression of the lung by long-standing oligohydramnios and by intrathoracic fluid, ascites, or intra-abdominal tumors of the fetus is the mechanism causing the acquired pulmonary hypoplasia. In this case, hypoplasia of the left lower pulmonary lobe is a congenital defect that originates in the embryonic period because, if it is induced by the compression, the entire left lung will be hypoplastic, considering the ultrasonographic findings.

The cause of fetal hydrothorax is almost unknown. However, the commonly mentioned pulmonary causes are cystic adenomatoid malformation of the lung, pulmonary lymphangiectasis, and pulmonary hypoplasia.[2] In this case, it seems reasonable to assume that the immaturity of the lymphatic system due to retardation in differentiation of the left lower pulmonary lobe induced the hydrothorax. This is supported by the fact that the fluid accumulation was observed only in the left side of the chest by ultrasound examination at the twenty-eighth week of pregnancy (Fig. 1).

Because of the large amount of fetal intrathoracic fluid, hydramnios in this case is thought to have been induced by difficulty in the fetal swallowing of amniotic fluid. This may also explain the lack of visualization of the gastrointestinal tract after intra-amniotic instillation of Urografin.

REFERENCES

1. Carrol B. Pulmonary hypoplasia and pleural effusions associated with fetal death in utero. Am J Roentgenol 1977;129:749-50.
2. Jouppila P, Kirkinen P, Herva R, Koivisto M. Prenatal diagnosis of pleural effusions by ultrasound. JCU 1983;11:516-9.

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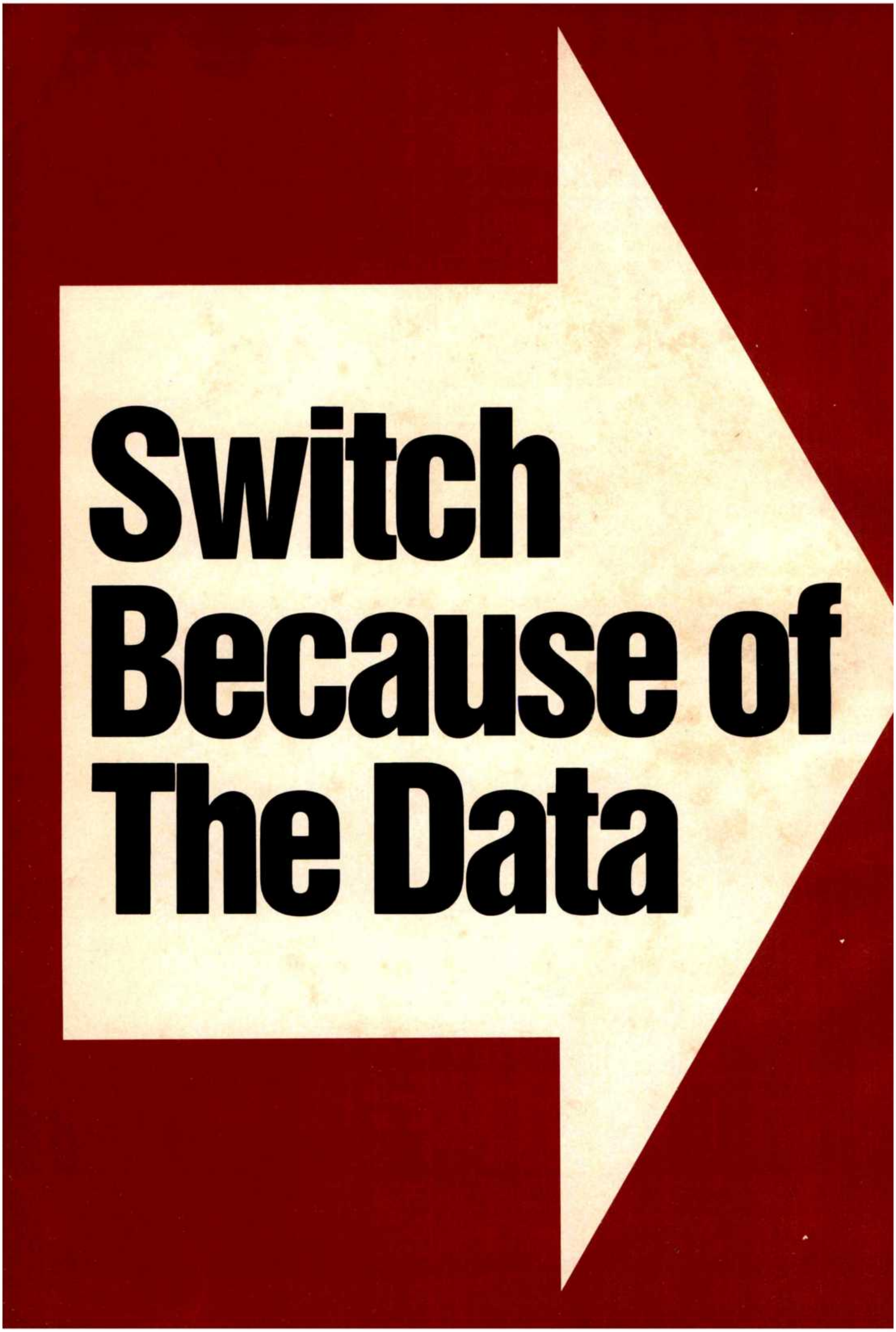

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Many infections caused by aerobic gram-negative organisms and by mixtures of organisms resistant to other cephalosporins, aminoglycosides, or penicillins respond to treatment with 'Cefizox'.

Because of the serious nature of some urinary tract infections due to *Ps. aeruginosa* and because many strains of *Pseudomonas* species are only moderately susceptible to 'Cefizox', higher dosage is recommended. Other therapy should be instituted if the response is not prompt.

Susceptibility studies on specimens obtained prior to therapy should be used to determine the response of causative organisms to 'Cefizox'. Therapy with 'Cefizox' may be initiated pending results of the studies; however, treatment should be adjusted according to study findings. In serious infections 'Cefizox' has been used concomitantly with aminoglycosides (see Precautions). Before using 'Cefizox' concomitantly with other antibiotics, the prescribing information for those agents should be reviewed for contraindications, warnings, precautions and adverse reactions. Renal function should be carefully monitored.

CONTRAINDICATIONS
'Cefizox' is contraindicated in persons who have shown hypersensitivity to cephalosporin antibiotics.

WARNINGS
BEFORE THERAPY WITH 'CEFIZOX' IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE WHETHER THE PATIENT HAS HAD PREVIOUS HYPERSENSITIVITY REACTIONS TO CEPHALOSPORINS, PENICILLINS, OR OTHER DRUGS. THIS PRODUCT SHOULD BE GIVEN CAUTIOUSLY TO PENICILLIN-SENSITIVE PATIENTS. ANTIBIOTICS SHOULD BE ADMINISTERED WITH CAUTION TO ANY PATIENT WHO HAS DEMONSTRATED SOME FORM OF ALLERGY, PARTICULARLY TO DRUGS. SERIOUS ACUTE HYPERSENSITIVITY REACTIONS MAY REQUIRE EPINEPHRINE AND OTHER EMERGENCY MEASURES.

***Pseudomembranous colitis* has been reported with the use of cephalosporins (and other broad-spectrum antibiotics); therefore, it is important to consider its diagnosis in patients w developed diarrhea in association with antibiotic use.** Treatment with broad-spectrum antibioti alters normal flora of the colon and may permit overgrowth of *Clostridia*. Studies indicate a toxin produced by *Clostridium difficile* is one primary cause of antibiotic-associated colitis. Cholestyramine and colestipol resins have been shown to bind the toxin *in vitro*. Mild cases o colitis may respond to drug discontinuance alone. Moderate to severe cases should be manag with fluid, electrolyte and protein supplementation as indicated. When the colitis is not relieved by drug discontinuance or when it is severe, oral vancomycin is the treatment of choice for an biotic-associated pseudomembranous colitis produced by *C. difficile*. Other causes of colitis should also be considered.

PRECAUTIONS
General: As with all broad-spectrum antibiotics, 'Cefizox' should be prescribed with caution i individuals with a history of gastrointestinal disease, particularly colitis.

Although 'Cefizox' has not been shown to produce an alteration in renal function, renal statu should be evaluated, especially in seriously ill patients receiving maximum dose therapy. As with any antibiotic, prolonged use may result in overgrowth of nonsusceptible organisms. Ca ful observation is essential; appropriate measures should be taken if superinfection occurs.

Drug Interactions: Although the occurrence has not been reported with 'Cefizox', nephrotoxicity has been reported following concomitant administration of other cephalosporins and aminoglycosides.

Pregnancy: (Category B.) Reproduction studies have been performed in rats and rabbits and have revealed no evidence of impaired fertility or harm to the fetus due to 'Cefizox'. There a however, no adequate and well-controlled studies in pregnant women. Because animal reprod tion studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Labor and Delivery: Safety of 'Cefizox' use during labor and delivery has not been establishe

Nursing Mothers: 'Cefizox' is excreted in human milk in low concentrations. Caution should exercised when 'Cefizox' is administered to a nursing woman.

Infants and Children: Safety and efficacy in infants from birth to six months of age have not bee established. In children six months of age and older, treatment with 'Cefizox' has been associa with transient elevated levels of eosinophils, SGOT, SGPT and CPK (creatine phosphokinase The CPK elevation may be related to I.M. administration.

ADVERSE REACTIONS
Ceftizoxime is generally well tolerated; the *most* frequent adverse reactions (*greater than* 1%, less than 5%) are: *Hypersensitivity*—Rash, pruritus, fever. *Hepatic*—Transient elevation in SG SGPT and alkaline phosphatase. *Hematologic*—Transient eosinophilia, thrombocytosis. Some individuals have developed a positive Coombs test. *Local*—Injection site—Burning, cellulitis, phlebitis with I.V. administration, pain, induration, tenderness, paresthesia.

Less frequent adverse reactions (*less than* 1%) are: *Hypersensitivity*—Numbness has been reported rarely. *Hepatic*—Elevation of bilirubin has been reported rarely. *Renal*—Transient ele tions of BUN and creatinine have been occasionally observed with ceftizoxime. *Hematologic* Anemia, leukopenia, neutropenia and thrombocytopenia have been reported rarely. *Genitourinary*—Vaginitis has rarely occurred. *Gastrointestinal*—Diarrhea; nausea and vomiti have been reported occasionally. Symptoms of pseudomembranous colitis can appear durin or after antibiotic treatment. (See Warnings.)

HOW SUPPLIED
Cefizox (sterile ceftizoxime sodium, FSK) is supplied in vials equivalent to 1 gram or 2 gram ceftizoxime; in "Piggyback" Vials for I.V. admixture equivalent to 1 gram or 2 grams of ceftizoxi

Cefizox (ceftizoxime sodium injection, FSK) as a frozen, sterile, nonpyrogenic solution in plastic containers—supplied in 50 ml. single-dose containers equivalent to 1 gram or 2 gram ceftizoxime, in 5% Dextrose (D5W). Do not store above -20°C.

'Cefizox' supplied as a frozen, sterile, nonpyrogenic solution in plastic containers is manufactu for Fujisawa SmithKline Corporation by Travenol Laboratories, Inc., Deerfield, IL 60015.

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References: 1. Lou, M.A., et al.: Comparative evaluation of ceftizoxime and cefoxitin in th treatment of intraabdominal infections, 14th Internat. Congr. Chemother., Kyoto, Japan, June 1985, pp. 2378-2379. **2.** Fu, K.P., and Neu, H.C., Antibacterial activity of ceftizoxi a ß-lactamase-stable cephalosporin, *Antimicrob. Agents Chemother.* 17:583-590, 1980 **3.** Thornsberry, C.: Review of in vitro activity of third-generation cephalosporins and othe newer beta-lactam antibiotics against clinically important bacteria, *Am. J. Med.* 79(Suppl. 14-20 (Aug.) 1985.

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A prospective evaluation of nipple stimulation techniques for contraction stress testing

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Nipple stimulation techniques for achieving a contraction stress test were evaluated prospectively in 1271 nipple stimulation contraction stress tests in 753 patients. Success was unrelated to parity, gestational age, or warm, moist towels but was related to the presence of spontaneous prestimulation contractions. Various stimulation techniques were equally successful in achieving a completed test in the presence of prestimulation contractions; however, continuous stimulation was more successful when contractions were absent. Hyperstimulation test results occurred in 21.5% of attempts and increased to 28.8% when bilateral, continuous stimulation was performed. (AM J OBSTET GYNECOL 1987;157:121-5.)

Key words: Contraction stress test, nipple stimulation, antepartum testing

After the initial report by MacMillan (see ref. 10) in 1980, the nipple stimulation contraction stress test has replaced the oxytocin-induced contraction stress test in many institutions. Obvious advantages in reduced cost and shortened testing times have been demonstrated.[1-3] Success at achieving a contraction stress test using nipple stimulation has been reported to vary between 57% and 100%, whereas test results and perinatal prognostic significance have reportedly been equivalent to oxytocin-induced contraction stress tests.[1, 4-6] Increased rates of uterine activity, both with and without late decelerations, have been described.[7-9]

Multiple stimulation techniques have been advocated in the literature.[1-7, 10] As originally reported, warm, wet towels were applied to the breasts, presumably to improve blood flow and, possibly, to influence oxytocin release. Others have advocated stimulation through the patient's clothing or stimulating the bare nipple. Unilateral and bilateral nipple stimulation, as well as intermittent and continuous stimulation techniques, have been employed.

The purpose of this report is to evaluate the ability of various stimulation techniques to achieve a successful test and to study the influence of gestational age, parity, and the presence of prestimulation contractions on successful test completion.

Material and methods

Both the University of California, Irvine Medical Center and St. Joseph Hospital are located in Orange, California. From March 1984 to August 1985, 491 patients at St. Joseph Hospital and 262 patients at University of California, Irvine Medical Center, who presented to their respective fetal diagnostic centers, were included in the study. All contraction stress test results were read by the same maternal-fetal medicine physicians. Patients were monitored for 15 minutes to observe baseline fetal heart rate and uterine activity before nipple stimulation. The six testing techniques were equally divided in each of the three gestational ages under study: <35 weeks, 35 to 41 weeks, and >41 weeks. The technique to be assigned was determined after the observation period from a random draw of sealed envelopes chosen for the appropriate gestational age. Random assignment of technique was repeated in a similar fashion for patients who underwent multiple tests. 1A, Dry towel over breasts with immediate unilateral, *continuous* stimulation throughout test. 1B, Dry towel over breasts with immediate *continuous* unilateral stimulation until onset of contraction and then *intermittent* stimulation—1 minute on, 1 minute off—until completion of test. 1C, Dry towel over breasts with immediate unilateral *intermittent* stimulation—1 minute on, 1 minute off—for duration of test.

Techniques 2A, 2B, and 2C were identical to the respective techniques described above except for the use of warm, moist towels applied to both breasts, with a 5-minute baseline before stimulation. Stimulation was by a rolling or tugging motion of the nipple through the towel. If a completed test did not appear imminent after approximately 30 minutes, the test was declared

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Table I. Indication for testing

Indication	*Patients (n = 753)*	*Tests (n = 1271)*
Diabetes	76	154
PIH	61	105
Hypertension	105	223
IUGR	129	215
Postdates	264	372
Other	184	310
Total	809*	1379*

PIH, pregnancy-induced hypertension; IUGR, intrauterine growth retardation.

*In some patients there was more than one indication for testing.

Table II. Gestational age and spontaneous prestimulation contractions

Gestational age (wk)	*Prestimulation contractions (n/%)*	*No prestimulation contractions (n/%)*
<35 (I)	38/28.8	94/71.2
35-41 (II)	460/50.5	451/49.5
>41 (III)	148/64.9	80/35.1

I versus II: $\chi^2 = 21.77$; $p < 0.0001$.
II versus III: $\chi^2 = 15.22$; $p < 0.001$.
I versus III: $\chi^2 = 43.69$; $p < 0.0001$.

a failure, and either an oxytocin-induced contraction stress test was performed (University of California, Irvine Medical Center) or bilateral continuous nipple stimulation (St. Joseph Hospital) was used. Patients who subsequently failed the bilateral continuous stimulation test received an oxytocin–induced contraction stress test.

A successful test result was defined as the presence of three uterine contractions within a 10-minute period, with each contraction lasting a minimum of 40 seconds. The interpretation of the nipple stimulation contraction stress test result was identical to that of an oxytocin–induced contraction stress test. Negative test results showed no decelerations throughout the test. Suspicious test results showed late or prolonged decelerations associated with <50% of contractions. A positive test result showed repetitive late decelerations with >50% of contractions. A hyperstimulation test result was defined as a prolonged contraction of >90 seconds accompanied by a late deceleration or frequent contractions with intervals of <2 minutes, accompanied with late decelerations.

Statistical analysis was performed by χ^2 and the Student t test. Multivariate analysis and logistic regression, using Bartholomew's method of χ^2, and the Mantel-Haenszel technique were employed where appropriate.

Results

All data results were analyzed separately by the hospitals, and no significant differences were noted. The data for both institutions were then combined. Unless otherwise specified, all of the results apply only to initial test attempts. A total of 1271 test attempts were performed on 753 patients for the indications listed in Table I.

Parity. A total of 611 (48.1%) test attempts were in nulliparous patients, and 660 (51.9%) attempts were in multiparous patients. Parity had no impact on achieving a completed test, the presence of prestimulation contractions, or the incidence of hyperstimulation test results.

Prestimulation contractions. Spontaneous prestimulation contractions were present in 651 of 1271 (51.2%) tests. The presence of prestimulation contractions increased significantly from 28.8% at <35 weeks to 50.5% at 35 to 41 weeks and 64.9% at >41 weeks ($p < 0.001$) (Table II). The presence of spontaneous prestimulation contractions significantly increased the likelihood of achieving a completed nipple stimulation contraction stress test.

Gestational age. A total of 133 (10.4%) test attempts were performed at <35 weeks, 910 (71.1%) attempts at 35 to 41 weeks, and 228 (17.9%) attempts at >41 weeks. The likelihood of achieving a completed nipple stimulation contraction stress test increased with advancing age. However, using the Mantel-Haenszel test, the increased likelihood of test success could be attributed to the increased frequency of spontaneous prestimulation contractions. In the absence of prestimulation contractions there was no significant difference in success rate in the three gestational age periods.

Test success and technique. There were 1271 initial nipple stimulation contraction stress test attempts, of which 923 (72.6%) were successful in obtaining a completed contraction stress test. Of the 348 test failures, 127 tests were completed by a standard contraction stress test technique. At St. Joseph Hospital, 164 patients underwent 221 test attempts using continuous, bilateral stimulation after an initial test failure. A successful test result was achieved in 69.7% of the patients, resulting in an overall nipple stimulation contraction stress test success rate of 92.2% for St. Joseph Hospital (Table III). However, for the purposes of this report, only initial test attempts were included for evaluation.

There was no difference in achieving test success using dry towels (technique 1) when compared with warm, moist towels (technique 2).

When prestimulation contractions were present, all test techniques showed similar success rates at achieving a completed contraction stress test. However, in the

Table III. Test results

	Test					
Patients	*Success (n/%)*	*Fail (n)*	*Negative (n/%)*	*Positive (n/%)*	*Suspicious (n/%)*	*Hyperstimulation (n/%)*
Initial (n = 753)	923/72.6	348	607/65.7	4/0.4*	114/12.4†	198/21.4‡
Secondary (n = 164)	154/69.7	67	79/51.0	3/1.9*	28/18.3†	44/28.8‡

*$\chi^2 = 4.7$; $p < 0.03$.
†$\chi^2 = 3.9$; $p < 0.05$.
‡$\chi^2 = 3.8$; $p < 0.05$.

Table IV. Success rate by technique and prestimulation contractions

Technique	*Prestimulation contractions (−)*	*% Success*	*Prestimulation contractions (+)*	*% Success*	*Total (T)*	*% Success*
1A	99/128	77.3	82/105	78.1	181/233	77.7
1B	67/99	67.7	71/90	78.9	138/189	73.0
1C	55/98	56.1	78/104	75.0	133/202	65.8
2A	64/92	73.5	111/129	86.0	175/221	79.2
2B	63/87	72.4	88/110	80.0	151/197	76.6
2C	54/112	48.2	91/117	77.8	145/229	63.3

1A (−) versus 1C (−) $\chi^2 = 11.46$; $p < 0.01$.
2A (−) versus 2C (−) $\chi^2 = 9.4$; $p < 0.003$.
2B (−) versus 2C (−) $\chi^2 = 11.78$; $p < 0.001$.
1A (T) versus 1 C (T) $\chi^2 = 7.53$; $p < 0.01$.
2A (T) versus 2C (T) $\chi^2 = 13.75$; $p < 0.0005$.
2B (T) versus 2C (T) $\chi^2 = 8.86$; $p < 0.003$.

absence of prestimulation contractions, continuous (As) or continuous-intermittent (Bs) stimulation was successful more frequently than was intermittent (Cs) stimulation (Table IV). Mean stimulation to initial contraction times and completed test times were calculated from completed tests with no prestimulation contractions. The mean stimulation to initial contraction time was 3.8 ± 2.9 minutes. The mean completed test times for the dry and wet techniques were 15.5 ± 5.5 minutes and 20.1 ± 6.4 minutes, respectively. These times were not significantly different when the 5-minute warm towel baseline period was excluded. Neither initial contraction nor completed test times were influenced by parity, gestational age, or stimulation technique. Ninety-six percent of all successful tests were completed in 30 minutes or less.

The incidence of negative, suspicious, positive, and hyperstimulation test results was 65.7%, 12.4%, 0.4%, and 21.4%, respectively. Bilateral continuous stimulation resulted in an increase in hyperstimulation, suspicious, and positive test results (Table III).

Hyperstimulation. Prolonged contractions >90 seconds were noted in 452 of 923 (49.0%) successful test results. Late decelerations were noted in association with 198 (43.8%) results and were interpreted as hyperstimulation test results. No late decelerations were noted in 254 (56.2%) tests and were interpreted as negative nipple stimulation contraction stress test results. Prolonged uterine contractions were significantly more likely to occur if prestimulation contractions were present ($p < 0.001$) (Table V).

In negative nipple stimulation contraction stress test results, without prestimulation contractions, the incidence of prolonged uterine contractions increased with gestational age, from 12.1% at <35 weeks to 48.1% at >41 weeks ($p < 0.01$) (Table V).

The overall rate of hyperstimulation test results was 21.5% and increased from 9.4% at <35 weeks to 29.0% at >41 weeks ($p < 0.001$) (Table VI).

Using the Mantel-Haenszel technique, there is a statistically significant association ($p < 0.0001$) between gestational age/prestimulation contractions and prolonged contractions during nipple stimulation. However, the ratio of late decelerations to prolonged contractions remained constant during all gestational ages, and the presence of prestimulation contractions was not associated with an increase in hyperstimulation test results. Furthermore, no correlation was noted between hyperstimulation test results and parity, stimulation technique, or warm, moist towels.

Table V. The incidence of prolonged contractions in negative test results with and without prestimulation contractions

	<35 Wk (I)		*35-41 Wk (II)*		*>41 Wk (III)*	
	Prolonged uterine contractions (n/%)	*Negative test (n)*	*Prolonged uterine contractions (n/%)*	*Negative test (n)*	*Prolonged uterine contractions (n/%)*	*Negative test (n)*
+ Uterine contractions	9/37.5	24	125/61.9	202	41/60.3	68
− Uterine contractions	4/12.1	33	62/36.3	171	13/48.1	27
Total	13/22.8	57	187/50.1	373	54/56.8	95

I + uterine contractions versus − uterine contractions: $\chi^2 = 6.54$; $p < 0.01$.
II + uterine contractions versus − uterine contractions: $\chi^2 = 24.25$; $p < 0.0001$.
I versus II: $\chi^2 = 13.03$; $p < 0.001$.
I versus III: $\chi^2 = 16.10$; $p < 0.001$.

Table VI. Hyperstimulation by gestational age with and without prestimulation contractions

	<35 Wk (I)		*35-41 Wk (II)*		*>41 Wk (III)*	
	Hyperstimulation (n/%)	*Completed NSCST (n)*	*Hyperstimulation (n/%)*	*Completed NSCST (n)*	*Hyperstimulation (n/%)*	*Completed NSCST (n)*
+ Uterine contractions	1/3.2	31	86/23.6	365	35/29.4	119
− Uterine contractions	8/12.3	65	54/18.9	286	16/28.1	57
Total	9/9.4	96	140/21.5	651	51/29.0	176

NSCST, Nipple stimulation contraction stress test.
I versus II: $\chi^2 = 7.69$; $p < 0.01$.
II versus III: $\chi^2 = 4.35$; $p < 0.05$.
I versus III: $\chi^2 = 13.83$; $p < 0.001$.

Comment

Various reports have evaluated serum oxytocin levels after nipple stimulation in the antepartum period.[11, 12] No consistent rise in oxytocin levels has been demonstrated. Despite this fact, nipple stimulation has been used effectively for cervical ripening, induction of labor, and antepartum testing.[1, 2, 5-7, 13]

Success rates of nipple stimulation contraction stress tests have been reported to be between 57% and 100%. Our overall success rate of 72.6% was similar to that reported previously at our institution by Oki et al.[4] The secondary success rate of 92.2% was similar to that reported by other investigators.[1, 2, 5-7]

The rate of success was not influenced by parity, gestational age, or warm, moist towels. Tests were more likely to be successful if prestimulation contractions were present, suggesting that the uterus was more receptive to the effects of nipple stimulation, by whatever mechanism. However, even in the absence of prestimulation contractions, 65% of all attempts were successful, with no significant difference noted by gestational age. This would suggest that nipple stimulation is a major determinant in achieving a successful test result, albeit the success rate improves when prestimulation contractions are present. The stimulation technique was unrelated to the achievement of a successful test result in the presence of prestimulation contractions. This suggests that if the uterus is sufficiently receptive, success is equally likely, irrespective of stimulation technique. In the absence of prestimulation contractions, continuous stimulation was more successful in achieving a completed test.

Previous investigators have found a higher incidence of prolonged uterine activity with the use of a nipple stimulation contraction stress test.[6] In our study group, 49.0% of the patients had prolonged contractions. No comparable information is available in the literature regarding the incidence of prolonged contractions, without decelerations, using oxytocin. Prolonged contractions were found to be correlated with the presence of prestimulation contractions, both of which increased significantly with advancing gestational age. Although the ratio of hyperstimulation test results to tests with prolonged contractions remained essentially constant with advancing gestational age, the number of tests with prolonged contractions increased significantly toward term, thereby accounting for the significant increase in hyperstimulation test results.

The incidence of hyperstimulation test results was particularly disturbing, because our protocol calls for repeat testing within 24 hours. This places an undue burden on the patient and the testing facility. It is entirely possible that our intermittent techniques (1B, C, 2B, and C) were physiologically closer to continuous stimulation than to intermittent stimulation. Our intermittent stimulation protocol was designed arbitrarily and when most preliminary studies were using continuous stimulation. Prolongation of the stimulation-free interval, as others have subsequently reported, may reduce the incidence of hyperstimulation test results.

Based on our data, a choice of nipple stimulation technique does not depend on parity, gestational age, or the use of warm, moist towels. Continuous stimulation seems to succeed more frequently in obtaining a completed test in the absence of prestimulation contractions but results in a high incidence of hyperstimulation test results when compared with other reports. More intermittent stimulation may reduce the incidence of hyperstimulation results; however, in the absence of prestimulation contractions it is also more apt to result in a higher number of test failures. Further refinements in nipple stimulation intervals appear warranted and might be based on the presence or absence of prestimulation contractions.

REFERENCES

1. Huddleston JF, Sutliff G, Robinson D. Contraction stress test by intermittent nipple stimulation. Obstet Gynecol 1984;63:699.
2. Chayen B, Scott E, Cheng C, Perera C, Schiffer M. Contraction stress test by breast stimulation as part of antepartum monitoring. Acta Obstet Gynecol Scand 1985;64:3.
3. MacMillan JB, Hale RW. Contraction stress testing with mammary self-stimulation. J Reprod Med 1984;29:219.
4. Oki EY, Keegan KA, Freeman RK, Dorchester WL. The nipple stimulated contraction stress test. (Submitted for publication.)
5. Copel JA, Otis CS, Stewart E, Rosetti C, Weiner S. Contraction stress testing with nipple stimulation. J Reprod Med 1985;30;465.
6. Hill WC, Moenning RK, Katz M, Kitzmiller JL. Characteristics of uterine activity during the breast stimulation stress test. Obstet Gynecol 1984;654:489.
7. Schellpfeffer MA, Hoyle D, Johnson JW. Antepartum uterine hypercontractility secondary to nipple stimulation. Obstet Gynecol 1985;65:588.
8. Viegas OA, Arulkumaran S, Gibb DM, Ratnam SS. Nipple stimulation in late pregnancy causing uterine hyperstimulation and profound fetal bradycardia. Br J Obstet Gynecool 1984;91:345.
9. Lenke RR, Nemes J. Use of nipple stimulation to obtain contraction stress test. Obstet Gynecol 1984;63:345.
10. MacMillan JB. The contraction stress test induced by maternal mammary self-stimulation. Presented at District VIII meeting of The American College of Obstetricians and Gynecologists, Colorado Springs, 1980.
11. Leake RD, Delbert DA, Fisher R, Buster JE. Oxytocin secretory response to breast stimulation in pregnant women. AM J OBSTET GYNECOL 1984;148:259.
12. Druzin M, Fuchs A, Fuchs F, et al. Serum oxytocin (OT) levels during contraction stress test (CST) induced by maternal nipple stimulation (MNS). Presented at the Thirtieth Annual Meeting of the Society for Gynecologic Investigation, March 17-20, 1983.
13. Elliot JP, Flaherty JF. The use of breast stimulation to ripen the cervix in term pregnancies. AM J OBSTET GYNECOL 1983;145:553.

Heart rate variation and movement incidence in growth-retarded fetuses: The significance of antenatal late heart rate decelerations

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In 37 intrauterine growth-retarded fetuses, combined 1-hour recordings of fetal heart rate and body movements were made within 24 hours of elective cesarean section. Fetal body movements were recorded simultaneously by use of real-time ultrasound. The study group was divided into two subgroups, according to the presence (n = 29) or absence (n = 8) of antepartum late heart rate decelerations. Correlations were made with umbilical blood gas values obtained immediately after cesarean section. Baseline heart rate variation was reduced below the normal range in 88% of the intrauterine growth-retarded fetuses with decelerations but in only 37% of the group without decelerations. A reduction in fetal heart rate accelerations and body movements and an increase in mean heart rate also were observed only in the group with decelerations. Late heart rate decelerations were associated with low Po_2 values in both umbilical artery and vein. It is concluded that in intrauterine growth-retarded fetuses reduced heart rate variation and movement incidence correlate with the presence of late heart rate decelerations before birth and with hypoxemia at birth. (Am J Obstet Gynecol 1987;157:126-33.)

Key words: Intrauterine growth retardation, fetal heart rate, fetal heart rate decelerations, fetal movements, fetal hypoxemia

With progressive deterioration of the fetal condition in utero, both heart rate variation and fetal movements decrease. Just before fetal death the baseline variability is decreased to less than 5 bpm, with no accelerations and with late decelerations, that is, "the terminal pattern." This condition is strongly associated with fetal acidemia.[1]

Recently with computerized analysis of heart rate records an association between reduced heart rate variation and growth retardation has been established in the human fetus. In this study late heart rate decelerations were usually present, but acidemia had not yet developed.[2] It was therefore concluded that the initial reduction of long-term heart rate variation is not caused by acidemia but is caused by some other factor associated with growth retardation. As heart rate variation and the incidence of body movements are reduced immediately after late heart rate decelerations it has been suggested that these reductions are associated with fetal hypoxemia.[3]

The aim of the present study is twofold. First it is an attempt to gain more insight as to when heart rate variation and movements decrease in the intrauterine growth-retarded (IUGR) fetus. Second, it is an attempt to examine the relationship of these parameters to acid-base status at delivery. In this article results based on 37 IUGR fetuses are presented, with combined recordings of fetal heart rate and fetal motility obtained within 24 hours of elective cesarean section.

Patients and methods

The study group consisted initially of 49 patients, admitted to the obstetric ward with a clinical diagnosis of intrauterine growth retardation. Excluded were infants delivered vaginally, infants with birth weights above the tenth percentile according to the Dutch (Kloosterman) growth curves, and infants with congenital malformations. Thirty-seven fetuses who had a weight at birth below the tenth percentile, corrected for sex, parity, and gestational age, remained in the study group. In the hospital Nonstress fetal heart rate monitoring was performed daily for a minimum duration of 45 minutes. The subjects were divided into two subgroups on the basis of presence or absence of late heart rate decelerations in any of the antenatal heart rate records. An example of a late heart rate deceleration is presented in Fig. 1. Fetuses with one or more late heart rate decelerations were designated as

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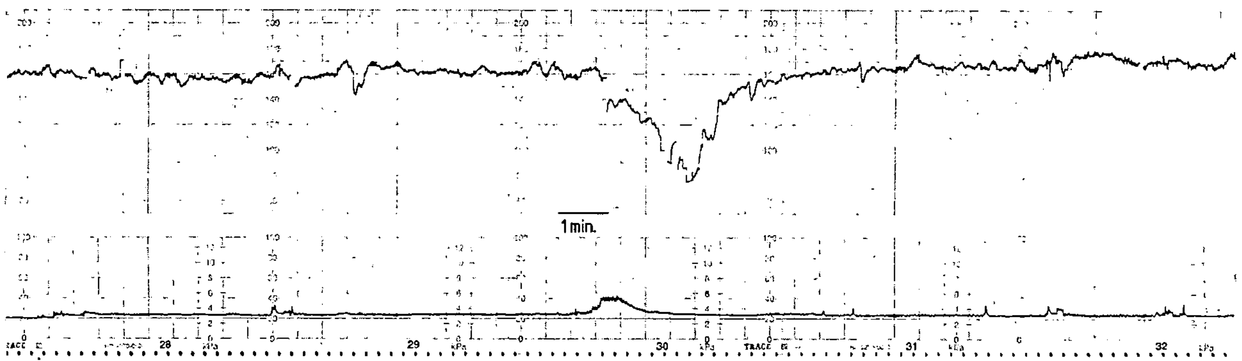

Fig. 1. An example of an antenatal late heart rate deceleration.

Table I. Clinical data in cases of IUGR fetuses

	Group with decelerations (n = 29)	*Group without decelerations (n = 8)*
Pregnancy-induced hypertension (No.)	19	4
Albuminuria (No.)	13	2
Antihypertensive drugs (No.)	5	0
Type 1 diabetes mellitus (No.)	2	0
Gestational age at birth (wk)		
Mean	33.3	35.9
Range	28-38	31-39
Birth weight (gm)		
Mean	1245	1611
Range	610-2210	950-2530
Birth weight percentile		
<2.3	15	4
2.3-5	8	1
5-10	6	3
>10	0	0
Stillbirth (No.)	1	0
Neonatal death (No.)	1	1
Intubation (No.)	5	1

the group with decelerations (n = 29). The remainder, who had normal or suboptimal heart rate traces but no detectable late heart rate decelerations, were designated as the group without decelerations (n = 8). All patients were delivered by elective cesarean section between 28 and 39 weeks of gestation. Clinical data of the patients are shown in Table I.

The combined fetal heart rate and movement recordings discussed in this paper were all made within 24 hours of cesarean section, 70% of which were within 6 hours. All recordings had a duration of 60 minutes and were made with the patient in a semirecumbent position, slightly tilted to the left. The fetal heart rate recordings were made with a Hewlett Packard cardiotocograph (Model 8030 A). Fetal pulse intervals were derived from an abdominal electrocardiographic signal (n = 15) or from a phonomagnetic signal (n = 22) and were stored on a minicassette after preprocessing by a microprocessor. The data were analyzed with a previously described computer program.[4] The baseline heart rate variation was calculated as the root mean square, after exclusion of accelerations and decelerations in excess of 40 msec (≈14 bpm). Only heart rate records with a fail time <70% were used in this study.[4] Three heart rate records had to be rejected for this reason. The fetal heart rate data from the growth-retarded fetuses were compared with similarly collected and processed data from 260 records made in 112 normal uncomplicated pregnancies between 28 and 40 weeks of gestation. Data on the majority of these pregnancies (n = 60) have been published before.[5] In making comparisons the effect of gestational age was taken into account.

Simultaneously with the recording of heart rate, fetal body and breathing movements were observed with a linear-array scanner (Aloka, Model SSD-256; Siemens, Imager 2300). The transducer was held in a parasagittal plane so that the fetal trunk and head were visible. Fetal body and breathing movements were recorded digitally by means of an event marker and encoded on the same tape as the fetal pulse intervals. Since the fetal body movements were analyzed in 3.75-second epochs, only

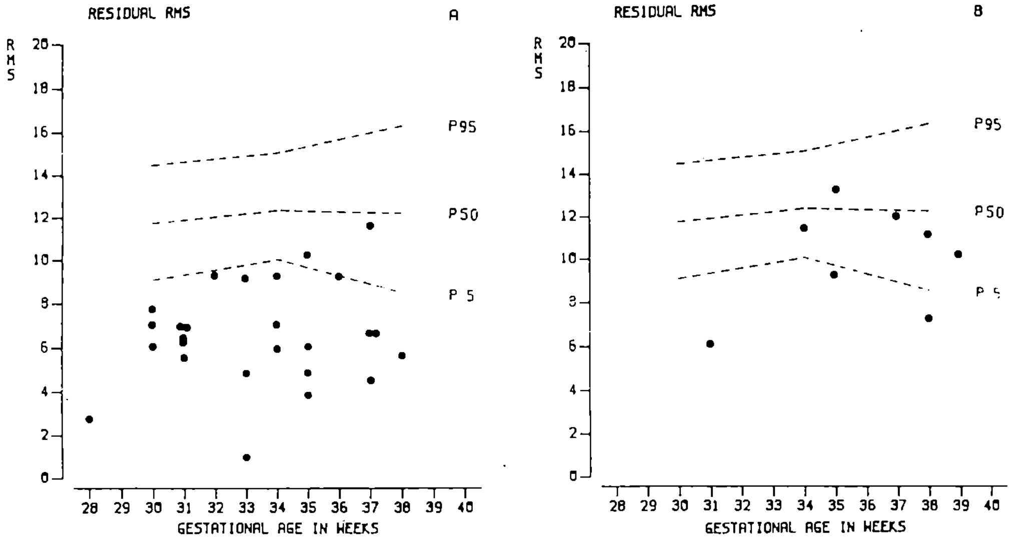

Fig. 2. Baseline heart rate variability (root mean square, *RMS*) of IUGR fetuses with decelerations *(A)* and of IUGR fetuses without decelerations *(B)*. The *dotted lines* indicate the percentiles of the control fetuses. Control data are based on 260 records made in 112 pregnancies.

one fetal movement was accepted each 3.75 seconds irrespective of the frequency of pressing the button of the event marker. Data on fetal body movements from 101 1-hour records obtained in 52 normal, uncomplicated pregnancies were used as controls. Pregnancy and fetal outcome in this group were uneventful. All infants were above the tenth percentile according to the Dutch (Kloosterman) curves, did well at birth, and did not need special pediatric attention. These data were collected together with more recent data on heart rate variation in normal pregnancies and have been published only in part.[6,7] Data on fetal breathing movements are not included in this paper.

The patients of the study group were delivered by elective cesarean section because of suspected fetal distress before the onset of labor, except for one infant that died in utero. Twenty-nine women were under general anesthesia; the remaining seven infants were delivered with the mothers under epidural analgesia. The general anesthesia consisted of preoxygenation with 100% oxygen at a flow rate of 10 L/min; thiopental sodium, 225 to 250 mg; suxamethonium chloride, 50 mg; and 0.5% halothane. The patients with epidural analgesia were also preoxygenated as mentioned above.

At delivery the umbilical cord was double clamped, and arterial and venous blood was analyzed immediately for pH, $\mathrm{P_{CO_2}}$, $\mathrm{P_{O_2}}$, and base excess, with the use of an automatic pH–blood gas analyzer (Model AVL 940). The acid-base and blood gas values of 45 infants delivered by elective cesarean section at 37 to 40 weeks of gestation were used as control values. Thirty of these infants were delivered with the mothers under general anesthesia; in the remaining 15 the mothers were under epidural analgesia. Anesthetic procedures were performed as described in the study group. All these infants had normal antenatal heart rate records and were above the twenty-fifth percentile, according to the Dutch (Kloosterman) curves. The indications for elective termination of pregnancy, before the onset of labor, were breech presentation (n = 16), cephalopelvic disproportion (n = 25), and poor obstetric history but normal pregnancy (n = 4). This control group was not part of the group in which the normal fetal heart rate pattern and body movement incidence were studied.

Statistical significance was determined with Student's *t* test.

Results

In the group with decelerations the baseline fetal heart rate variation assessed numerically was below the fifth percentile of the normal range in 23 of the 26 cases (88%) (Fig. 2). In IUGR fetuses without antenatal decelerative heart rate traces baseline heart rate variability was reduced in three of the eight cases (37%) (Fig. 2). Heart rate variation in the group with decelerations was significantly reduced compared with that in the group without decelerations and the control group ($p < 0.005$; Table II). This reduction was also present when gestational age was taken into account.

The mean heart rate was significantly increased in the group with decelerations compared with the control group, although it was usually still within the normal range ($p < 0.005$; Table II). The mean heart rate of

Table II. Baseline heart rate variability, mean heart rate, number of accelerations, and gross body movements of the growth-retarded and control fetuses

	IUGR group			
Gestational age (wk)	*With decelerations*	*Without decelerations*	*Combined*	*Control group*
Baseline heart rate variability (root mean square in msec)				
28-32	6.7 ± 0.6 (10)	6.9 (1)	6.7 ± 0.5 (11)	11.9 ± 0.2 (48)
33-36	6.5 ± 0.9 (11)	11.3 ± 1.1 (3)	7.6 ± 1.1 (14)	12.3 ± 0.2 (80)
37-40	7.0 ± 1.2 (5)	10.2 ± 1.0 (4)	8.4 ± 0.9 (9)	12.3 ± 0.2 (132)
Combined (28-40)	6.9 ± 0.5 (26)	10.2 ± 0.8 (8)	7.5 ± 0.5 (34)	12.3 ± 0.2 (260)
Mean heart rate (bpm)				
28-32	148.1 ± 2.9 (10)	157.7 (1)	149.0 ± 3.3 (11)	141.5 ± 0.9 (48)
33-36	147.1 ± 3.0 (11)	140.5 ± 3.3 (3)	145.7 ± 2.5 (14)	141.0 ± 0.8 (80)
37-40	146.4 ± 1.2 (5)	136.3 ± 4.1 (4)	141.9 ± 2.5 (9)	141.0 ± 0.8 (132)
Combined (28-40)	147.4 ± 1.7 (26)	140.6 ± 3.4 (8)	145.8 ± 1.6 (34)	141.1 ± 0.5 (260)
No. of accelerations (>40 msec and >15 sec) per hour				
28-32	0.1 ± 0.1 (10)	0.0 (1)	0.1 ± 0.1 (11)	4.4 ± 0.8 (48)
33-36	0.4 ± 0.4 (11)	7.3 ± 5.0 (3)	1.9 ± 1.2 (14)	11.8 ± 0.9 (80)
37-40	0.8 ± 0.6 (5)	10.5 ± 4.3 (4)	5.1 ± 2.5 (9)	14.4 ± 0.7 (132)
Combined (28-40)	0.4 ± 0.2 (26)	8.0 ± 2.9 (8)	2.2 ± 0.9 (34)	11.7 ± 0.5 (260)
Gross body movements (% of time)				
28-32	4.6 ± 0.9 (12)	6.9 (1)	4.8 ± 0.8 (13)	14.5 ± 0.8 (26)
33-36	7.6 ± 1.9 (12)	12.9 ± 3.5 (3)	8.7 ± 2.2 (15)	12.6 ± 0.9 (26)
37-40	4.4 ± 0.6 (5)	16.1 ± 6.8 (4)	9.6 ± 3.5 (9)	17.6 ± 1.2 (49)
Combined (28-40)	5.8 ± 0.9 (29)	13.7 ± 3.6 (8)	7.5 ± 1.2 (37)	15.7 ± 0.8 (101)

Values are mean ± SEM. Number of records is in parentheses.

the IUGR fetuses of the group without decelerations was not significantly different from that of the control group.

The number of accelerations per hour exceeding 40 msec (≈14 bpm) and lasting more than 15 seconds were significantly reduced in the group with decelerations ($p < 0.005$; Table II). The group without decelerations had accelerations within the normal range (Table II). This is in accordance with the fetal movement incidence in both groups. The lower limit (fifth percentile) of normal fetal activity was rather constant throughout the last trimester (Fig. 3). A reduction in movement incidence below the fifth percentile of the normal group occurred in 69% of the fetuses of the group with decelerations whereas in only two of the eight cases (25%) of the group without decelerations were fetal movements slightly reduced (Fig. 3). The combined growth-retarded group moved significantly less than the control fetuses ($p < 0.005$; Table II). As indicated, this is due to the reduction in movement incidence in the group with decelerations (Table II).

In Figs. 4 and 5, pH and Po_2 values in the umbilical artery and vein are shown. In most of the 36 IUGR fetuses (72%) umbilical artery pH was above 7.20. The mean arterial and venous pH values of the group with decelerations were lower than those of the control group ($p < 0.05$) even though most values were within the normal range (Fig. 4). In contrast, Po_2 values in both umbilical artery and vein were remarkably low in the group with decelerations (Fig. 5). Differences be-

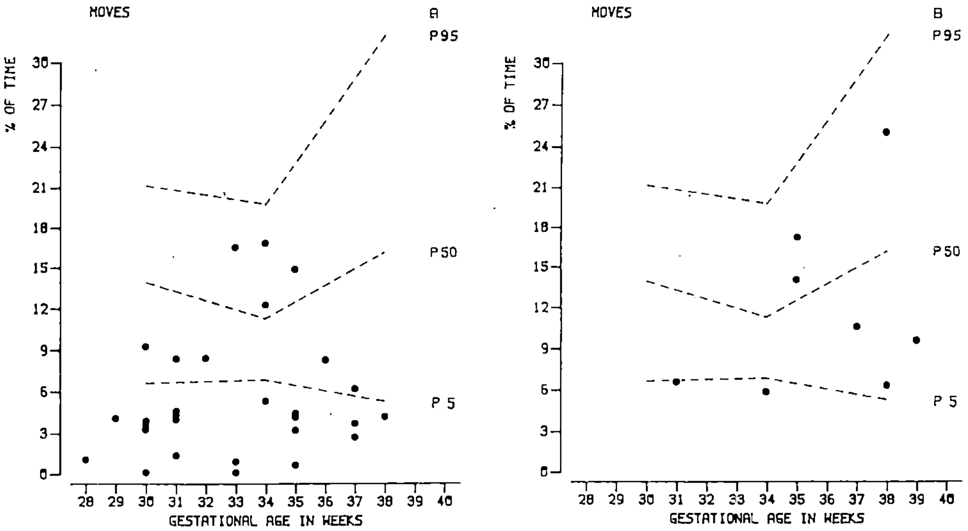

Fig. 3. Fetal body movements (percent of time) of IUGR fetuses with decelerations *(A)* and IUGR fetuses without decelerations *(B)*. The *dotted lines* indicate the percentiles of the control fetuses. Control data are based on 101 records made in 52 pregnancies.

tween this group and the group without decelerations and the control group were highly significant ($p < 0.005$). No significant differences were found in Pco_2 and base excess values between either the growth-retarded group or the control group. Table III summarizes all the blood gas values. To exclude possible effects of pH changes on oxygen tension, umbilical artery Po_2 values of IUGR fetuses with an umbilical artery pH between 7.20 and 7.28 were compared with those of control group fetuses whose umbilical artery pH values were in the same range. Again a significant difference in Po_2 was found ($p < 0.005$; Table IV).

To analyze the association between reduced baseline heart rate variability and low oxygen tension, IUGR fetuses with low and high heart rate variability were compared. A cutoff point was chosen at a baseline variability of 8 msec, as this was approximately 2 SD below the mean baseline heart rate variability. IUGR fetuses with a variability of <8 msec ($n = 21$) had significantly lower arterial and venous Po_2 values than those with variability >8 msec ($n = 12$) ($p < 0.05$). The group with a very low baseline heart rate variation included 19 (76%) of the IUGR fetuses with late heart rate decelerations. Only two (25%) of the IUGR fetuses without decelerations had a baseline heart rate variation below 8 msec. No differences existed in umbilical artery and vein pH and base excess. Pco_2 values were significantly higher in the group with the lowest heart rate variability ($p < 0.05$; Table V).

Comment

This study confirms earlier findings[2,8] that in growth-retarded fetuses long-term heart rate variation (that is, baseline variation and variation caused by accelerations and decelerations) is usually low. However, in the absence of late heart rate decelerations, baseline variation is nearly always within the normal range.[8] In the presence of late decelerations, however, baseline variation and the incidence of accelerations are reduced. These data imply that before late decelerations appear, the heart rate variation pattern of the growth-retarded fetus cannot be distinguished from that of the noncompromised fetus. Consequently reduction of long-term variation has to be considered a rather late sign of fetal compromise. At this stage of deterioration fetal acidemia has not yet developed, as 80% of the fetuses with reduced variation had an umbilical artery pH ≥7.20. This confirms the findings of Henson et al.[2] that the initial reduction of heart rate variation is not caused by acidemia. However, remarkably low Po_2 values in both umbilical artery and vein were found in the IUGR fetuses with decelerations. In this group hypoxemia was consistently present at elective cesarean section, in contrast to findings in IUGR fetuses without decelerations in whom Po_2 values were within the normal range. As the lowest Po_2 values were found in fetuses with reduced heart rate variation it is tempting to assume an association between chronic fetal hypoxemia and the reduction of heart rate variation.

Just before fetal death heart rate variation is usually extremely low and decelerations, mostly shallow in character, follow each Braxton Hicks' contraction. With this "terminal" pattern acidemia is present in 70% of the cases at cesarean section.[1] These late decelerations differ from those found in growth-retarded fetuses in whom only hypoxemia is present. With the "termi-

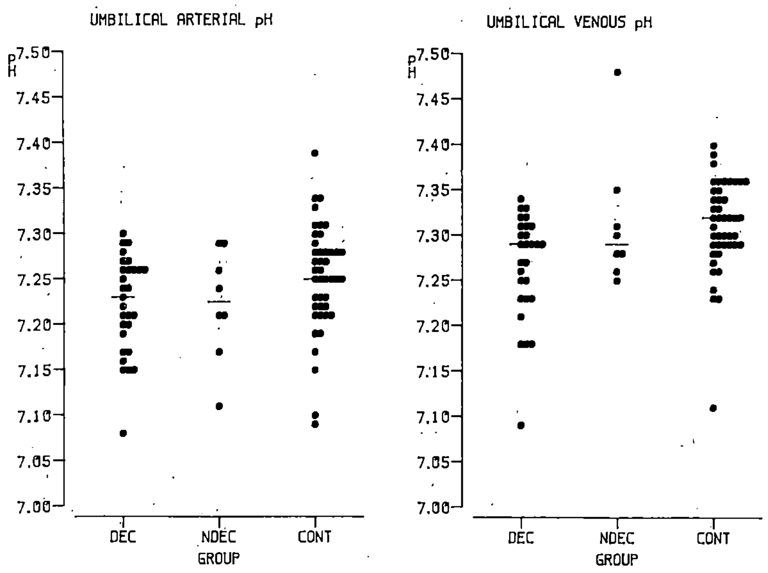

Fig. 4. Umbilical artery and vein pH values of IUGR fetuses with decelerations, IUGR fetuses without decelerations, and control fetuses.

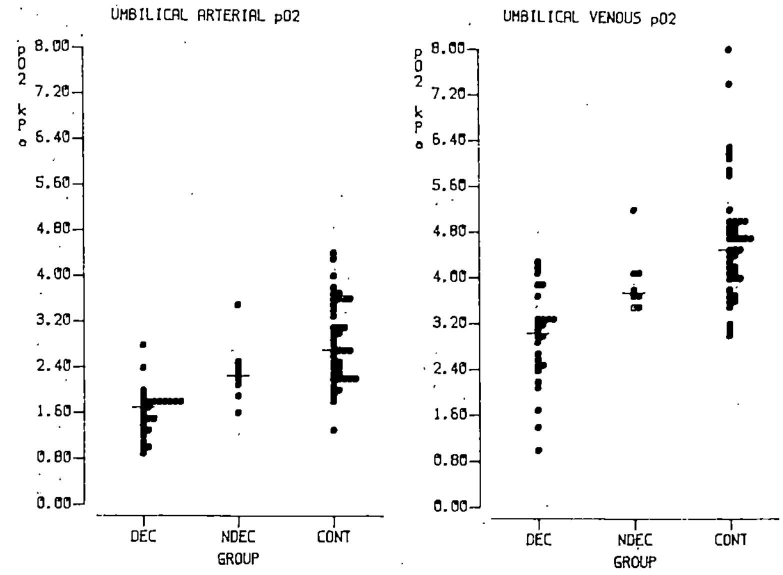

Fig. 5. Umbilical artery and vein P_{O_2} values of IUGR fetuses with decelerations, IUGR fetuses without decelerations, and control fetuses (kPa).

nal" pattern, variation is low during the deceleration, whereas in the group with decelerations an increase in variation is often found (Fig. 1). It may well be that the etiologic backgrounds of these two types of decelerations are different. Court and Parer[9] have described two possible mechanisms. The first mechanism is increased vagal activity due to chemoreceptor activation without reduced myocardial oxygen consumption. The increased heart rate variation often seen during these decelerations may be caused by simultaneously increased β-adrenergic receptor activity. The second mechanism occurs in terminal patterns, where hypoxic myocardial depression predominates over increased vagal activity. In these cases heart rate variation during the deceleration is reduced.

The present findings agree with those of Murata et al.,[10] who found that late decelerations appeared first in rhesus monkeys with progressive deterioration in fetal condition. This condition, which was associated with fetal hypoxemia, was followed by a decrease in accelerations (and in movements?) and a gradual decline in the pH. Reduced P_{O_2} values found in fetal

Table III. Blood gas values (mean ± SD) of IUGR fetuses and control fetuses at elective cesarean section

	IUGR group		
	With decelerations (n = 28)	*Without decelerations (n = 8)*	*Control group (n = 45)*
pH			
Umbilical artery	7.22 ± 0.05*	7.22 ± 0.06	7.25 ± 0.06
Umbilical vein	7.27 ± 0.06*	7.31 ± 0.07	7.31 ± 0.05
Po_2 (kPa)			
Umbilical artery	1.61 ± 0.42†‡	2.29 ± 0.55	2.81 ± 0.76
Umbilical vein	2.95 ± 0.83†‡	3.96 ± 0.56	4.66 ± 1.09
Pco_2 (kPa)			
Umbilical artery	7.68 ± 1.27	7.08 ± 0.83	7.30 ± 1.30
Umbilical vein	6.66 ± 1.27	5.89 ± 0.31	5.92 ± 0.90
Base excess			
Umbilical artery	−6.42 ± 3.45	−7.00 ± 3.05	−4.89 ± 3.38
Umbilical vein	−5.74 ± 3.01	−5.73 ± 2.22	−4.01 ± 2.01

*Group with decelerations versus control group: $p < 0.05$.
†Group with decelerations versus group without decelerations: $p < 0.005$.
‡Group with decelerations versus control group: $p < 0.005$.

Table IV. Comparison of Po_2 values of IUGR fetuses with decelerations and control fetuses with umbilical artery pH values in the same range (mean ± SD)

Po_2	*IUGR group with decelerations (n = 17) 7.20 < umbilical artery pH < 7.28*	*Control group (n = 25) 7.20 < umbilical artery pH < 7.28*
Umbilical artery	1.55 ± 0.38	2.69 ± 0.64*
Umbilical vein	3.27 ± 0.56	4.21 ± 0.90*

*$p < 0.005$.

sheep in which the endometrial caruncles have been removed also confirm the relationship between growth retardation and chronic hypoxemia.[11] The fetus can tolerate moderate hypoxia, in part by a reduction in oxygen consumption.[12] On the other hand, raised hematocrit and increased oxygen extraction[12] are other compensatory mechanisms that occur when oxygen delivery decreases. The mechanisms and pathways underlying reduction in heart rate variation still remain obscure. Suppression of the activity of medullary centers by endogenous opiates, such as β-endorphin, is one of the possibilities to which attention has recently been drawn.[13]

Estimating the respiratory status of the fetus in utero by means of the acid-base status at cesarean section has to be done very cautiously. The effects of anesthesia and surgical intervention must not be underestimated, yet the differences in Po_2 found in this study were so profound that we predict they will be confirmed if precise assessment of the antenatal respiratory status in the human is performed. With the rapid development of the umbilical cord blood sampling technique, under ultrasound guidance, this will be possible in the very near future.

Table V. Comparison of blood gas values of IUGR fetuses with lower and higher heart rate variability; cutoff point root mean square of 8 msec (mean ± SD)

	Root mean square <8 (n = 21)	*Root mean square >8 (n = 12)*
pH		
Umbilical artery	7.23 ± 0.04	7.23 ± 0.06
Umbilical vein	7.28 ± 0.04	7.31 ± 0.06
Po_2		
Umbilical artery	1.65 ± 0.49	2.11 ± 0.60*
Umbilical vein	3.24 ± 0.64	3.81 ± 0.61*
Pco_2		
Umbilical artery	7.91 ± 0.83	7.03 ± 0.92*
Umbilical vein	6.57 ± 0.73	5.87 ± 0.56*
Base excess		
Umbilical artery	−5.48 ± 3.08	−6.96 ± 3.44
Umbilical vein	−5.04 ± 2.55	−6.84 ± 4.31

*$p < 0.05$.

The increased mean heart rate in IUGR fetuses with late decelerations has, to our knowledge, not been described before. As the number of accelerations is reduced, it must be concluded that the increase in mean heart rate is due to an increase in baseline heart rate. Intrapartum tachycardia is, however, a well-known phenomenon of fetal compromise, and Dalton et al.[14] have reported higher basal fetal heart rates in hypertensive pregnancies. In the control fetuses mean heart rate did not decrease during the third trimester (Table II). This has been shown before and can be explained by an increase of heart rate accelerations (number and size) with gestational age, with a simultaneous fall in basal heart rate.[5]

In growth-retarded fetal sheep mean heart rates were also reported to be higher in the basal condition than those of control animals, probably because of the increased levels of catecholamines.[15] These findings suggest that the fetus adapts to chronic compromise by making changes in the cardiovascular system. One mechanism of adaptation is that of an increase of the basal heart rate.

In control fetuses the mean percentage of time fetal movements were present was consistent throughout the last trimester. No decline in mean activity was noted at the end of pregnancy. The normal range of fetal activity was wide, with a marked increase in range after 36 weeks of gestation, probably because of the development of behavioral states.[16] The fifth percentile of movement incidence was, however, rather stable. Therefore a well-chosen cutoff point in the normal fetal movement incidence might be of clinical value in the detection of the fetus at risk. In most IUGR fetuses without late heart rate decelerations the movement incidence was within the normal range. In IUGR fetuses with decelerations the quantity of motor output was reduced. However, approximately 30% of these fetuses had a movement incidence within the normal range. This implies that while a marked reduction of movements is a sinister sign, a quantitatively normal motor output does not guarantee fetal well-being. The reduction of fetal movements in growth retardation has also been reported by other investigators, mostly using nonimaging methods.[17] Chronic hypoxemia might account for this reduction of fetal activity. The intermittent occurrence of acute hypoxemia is another possibility, since it has been reported that superimposed acute hypoxemic events, as indicated by late heart rate decelerations, abolish or reduce fetal body movements in the human fetus.[3] In fetal sheep acute experimental hypoxia also reduces fetal trunk and limb movements.[18, 19]

From the present study it can be concluded that in IUGR fetuses reductions in heart rate variation and motility occur before the development of acidemia and that these changes are associated with late heart rate decelerations and fetal hypoxemia. In general a growth-retarded fetus will be found to have normal heart rate and motility pattern. It is not growth retardation that is detected by these assessment techniques but presumably the effects of chronic fetal hypoxemia.

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REFERENCES

1. Visser GHA, Redman CWG, Huisjes HJ, Turnbull AC. Nonstressed antepartum heart rate monitoring: implications of decelerations after spontaneous contractions. AM J OBSTET GYNECOL 1980;138:429.
2. Henson GL, Dawes GS, Redman CWG. Antenatal fetal heart rate variability in relation to fetal acid-base status at caesarean section. Br J Obstet Gynaecol 1983;90:516.
3. Bekedam DJ, Visser GHA. Effects of "hypoxemic events" on breathing, body movements, and heart rate variation: a study in growth-retarded human fetuses. AM J OBSTET GYNECOL 1985;153:52.
4. Dawes GS, Visser GHA, Goodman JDS, Redman CWG. Numerical analysis of the human fetal heart rate: the quality of ultrasound records. AM J OBSTET GYNECOL 1981; 141:43.
5. Visser GHA, Dawes GS, Redman CWG. Numerical analysis of the normal human antenatal fetal heart rate. Br J Obstet Gynaecol 1981;88:792.
6. Visser GHA, Zeelenberg HJ, De Vries JIP, Dawes GS. External physical stimulation of the human fetus during episodes of low heart rate variation. AM J OBSTET GYNECOL 1983;145:579.
7. Mulder EJH, Visser GHA. Braxton Hicks' contractions and motor behavior in the near-term human fetus. AM J OBSTET GYNECOL 1987;156:543.
8. Visser GHA. Antenatal cardiotocography in the evaluation of fetal well-being. Aust NZ J Obstet Gynaecol 1984;24:80.
9. Court DJ, Parer JT. Experimental studies of fetal asphyxia and fetal heart rate interpretation. In: Nathanielsz PW, Parer JT, eds. Research in perinatal medicine. Ithaca, New York: Perinatology Press, 1984:113.
10. Murata Y, Martin CB, Ikenoue T, et al. Fetal heart rate accelerations and late decelerations during the course of intrauterine death in chronically catheterized rhesus monkeys. AM J OBSTET GYNECOL 1982;144:218.
11. Robinson JS, Kingston EJ, Jones CT, Thorburn GD. Studies on experimental growth retardation in sheep: the effect of the removal of endometrial caruncles on fetal size and metabolism. J Dev Physiol 1979;1:45.
12. Owens JA, Alotta E, Falconer J, Robinson JS. Effect of restricted placental growth upon oxygen and glucose delivery to the fetus. In: Jones CT, Nathanielsz PW, eds. The physiological development of the fetus and newborn. London: Academic Press, 1985:33.
13. Hofmeyr GJ, Bamford OS, Dawes GS, Parkes MJ. High frequency heart rate variability in the fetal lamb. In: Jones CT, Nathanielsz PW, eds. The physiological development of the fetus and newborn. London: Academic Press, 1985:439.
14. Dalton KJ, Dawson AJ, Newcombe RG. Fetal heart rate changes in hypertensive pregnancies. In: Jones CT, Nathanielsz PW, eds. The physiological development of the fetus and newborn. London: Academic Press, 1985:753.
15. Robinson JS, Jones CT, Kingston EJ. Studies on experimental growth retardation in sheep: the effects of maternal hypoxemia. J Dev Physiol 1983;5:89.
16. Nijhuis JG, Prechtl HFR, Martin CB Jr, Bots RSGM. Are there behavioural states in the human fetus? Early Hum Dev 1982;6:177.
17. Jarvis GJ, MacDonald HN. Fetal movements in small-for-dates babies. Br J Obstet Gynaecol 1979;86:724.
18. Natale R, Clewlow F, Dawes GS. Measurement of fetal forelimb movements in the lamb in utero. AM J OBSTET GYNECOL 1981;140:545.
19. Martin CB Jr, Voermans TMG, Jongsma HW. Effect of hypoxia on trunk movements in fetal sheep. In: Proceedings of the thirty-second annual meeting of the Society for Gynecologic Investigation, Phoenix, Arizona, March 20-23, 1985:169.

Transabdominal chorionic villus sampling: A freehand ultrasound-guided technique

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A simple transabdominal chorionic villus sampling method, carried out with a 20-gauge spinal needle under ultrasound guidance, was evaluated in 100 cases selected for genetic evaluation in the first trimester. Its use was limited to the management of anatomic and clinical conditions that contraindicated transcervical aspiration. The high efficacy of the method was demonstrated by an ability to obtain enough villus tissue for karyotyping in all but one case. In 94% of the cases, only one pass of the needle was required. Although the only complication observed was light bleeding in four cases, the safety of the method needs more extensive evaluation. (AM J OBSTET GYNECOL 1987;157:134-7.)

Key words: Chorion biopsy, transabdominal needling, ultrasound

The ability to detect fetal genetic diseases by chorionic tissue analysis has resulted in a great number of sampling methods. Four main approaches are being used. In three of them, the catheter,[1] biopsy forceps,[2] or endoscope[3] is inserted via the cervical canal; in the fourth method, the aspiration is done by transabdominal puncture.[4] Most experience has been accumulated with transcervical catheter (Trophocan, Portex Inc., Wilmington, Maine) aspiration, a method that has proved to be highly efficient and relatively safe.[5]

Some anatomic and clinical conditions have been recognized as being unfavorable for safe and successful transcervical sampling; therefore the feasibility of a simple transabdominal ultrasound-guided chorionic villus sampling (CVS) method has been evaluated and is described in this article.

Patients

The 100 cases in the present series were selected for transabdominal CVS because of absolute or relative contraindications to the transcervical route (Table I). CVS was performed at 8, 9, 10, 11, 12, 13, and 14 weeks in 4, 31, 30, 26, 6, 2, 1 cases, respectively. Maternal age was more than 35 years in 77% of the cases. The indication for first-trimester diagnosis was chromosome determination in 96 cases, metabolic disease in one case, fetal sex determination for X-linked disease in two cases, and deoxyribonucleic acid analysis for hemophilia A in one case. The placenta was anterior in 66% of cases and posterior in the remaining 34%.

Table I. Conditions of absolute or relative contraindication to transcervical aspiration, in which a transabdominal sampling should be advised

- Vaginismus*
- Vaginitis*
- Inaccessible cervical canal*
- Pronounced angle of uterine corpus on cervix†
- Multiple pregnancy†
- ≥12 weeks of gestation†
- Previous failure of transcervical sampling†

*Absolute.
†Relative.

Methods

A freehand aspiration was achieved through the maternal abdominal wall with a 20-gauge, 90 mm spinal needle with sector scan guidance at 5 MHz (Sigma 1SC, Kontron Instruments Inc., Everett, Massachusetts) (Fig. 1, *A*). The placenta was identified and its depth calculated. A compromise was made between the extension and thickness of the placenta and its distance from the maternal wall. Bladder emptying or filling was sometimes required to obtain a more favorable position of the uterus. The skin was sterilized with topical application of povidone iodine and the area draped in a sterile fashion. Because the transducer was 5 to 10 cm away from the insertion site, a nonsterilely dressed person could hold the scanner; neither sterile gloves nor sterile contact gel was needed. The needle was introduced under continuous ultrasonic surveillance without local administration of anesthetics. When the tip was clearly visible within the placenta (Fig. 1, *B* and *C*), the stylet was replaced by a 20 ml Luer-Lok syringe containing 3 ml of Henk's solution. The chorionic tissue was then aspirated by combining repeated strong suc-

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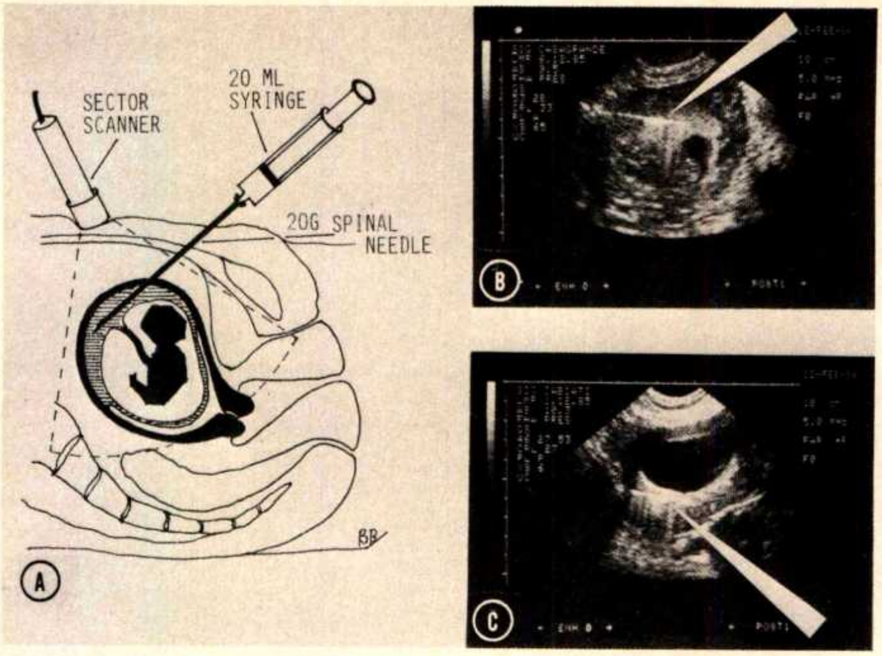

Fig. 1. Diagram of transabdominal chorion biopsy by 20-gauge spinal needle under ultrasound guidance *(A)*. Ultrasound monitor shows needle *(arrow)* sampling chorionic villi in two cases at 10 weeks *(B)* and 9 weeks *(C)* with an anterior and a posterior placenta, respectively.

tions (equivalent to about 20 ml depression) and a slow backward-and-forward movement of the needle tip. No more than two needle insertions were performed. Neither premedication nor postsampling care was required.

Results

We failed to obtain chorionic tissue in six of the 100 cases with the first attempt, but tissue was obtained in these cases with a second insertion performed during the same session. The failures were apparently not related to the gestational age or to the placental location. The majority, five out of six cases, occurred in the first 30 cases, and the difficulties most likely represented inadequate technical experience. In a twin pregnancy with two separate placentas, chorionic tissue was easily obtained by cervical aspiration from one placenta, but the second placenta, located far from the internal cervical os, could be sampled only through the abdominal wall.

The distribution of the weight of villus tissue obtained with a single aspiration is reported in Table II. For comparison, the villus weight distribution of 200 cases of transcervical aspiration is provided. Although the ranges of the weight distribution are quite similar for both techniques, the transabdominal aspirations more frequently produced samples of <5 mg, whereas the transcervical samples were frequently ≥ 50 mg. The samples of <5 mg were more frequently obtained from anterior (14/66) than from posterior (2/34) placentas.

Five pregnancies were terminated by elective abortion (Table III); in three, a fetal aneuploidy was diagnosed by direct karyotyping,[6] and two fetuses at risk for X-linked disease were male. No fetal losses were observed at the time of writing in the 95 continuing pregnancies; 68% of these are currently more than 20 weeks of gestation. In four cases, bleeding or spotting was reported after CVS. Delivery has occurred in 17 cases; all were at term with birth weights between the tenth and ninetieth percentiles.

Comment

Although the experience reported in large series seems conclusive with respect to the efficacy of the various chorionic villus sampling techniques,[5] no reliable clinical trials comparing the different approaches are yet available. While randomized trials between transcervical and transabdominal aspiration are awaited,[5] it is of practical interest to evaluate the suitability of the transabdominal puncture method in conditions unfavorable for the transcervical approach. In addition, we wanted to explore means of reducing the invasiveness and relative complexity of transabdominal sampling, as previously described.[4, 8]

The new sampling procedure was highly successful, without regard to the placental location or gestational age. An overall success rate of 99% was obtained; a second puncture was required in only 6% of cases. Although the weight distribution of the aspirated specimens points to a shift toward smaller samples, in 95%

Table II. Weight distribution of chorionic tissue specimens obtained by transabdominal or transcervical aspiration methods

Chorionic villus sampling method	*% Specimens obtained according to weight*					
	<5 mg	*5-10 mg*	*11-20 mg*	*21-50 mg*	*51-100 mg*	*>100 mg*
20-gauge spinal needle	17	35	19	27	2	—
Trophocan	1	16	15	45	22	1

Table III. Clinical data of 100 consecutive chorionic villus sampling cases obtained by a transabdominal aspiration

Total No. of cases	100
Twin pregnancies	1
Successful sampling	100
At first aspiration	94
Selective abortion	5
Concluded pregnancies	17
Early complications	
Bleeding	1
Spotting	3
Fetal loss	0

of the last 40 cases, the amount of chorionic tissue obtained by a single insertion exceeded 10 mg. By this method it is possible, with two needle insertions, to provide a sufficient amount of tissue for successful deoxyribonucleic acid analysis in complicated diagnostic conditions. In only one case was fetal karyotyping not possible because of insufficient villi.

The success of sampling was dependent on reliable needle tip detection and obtaining an adequate course through the placental substance. In fact, with poor ultrasound visualization, one may fail to enter the placenta, or, if the needle is placed in too thin a portion of the placenta, inadequate movement of the tip precludes the aspiration of a sufficient sample.

Transabdominal CVS, as carried out in the present experience, has some advantages when compared with the previously described technique, in which a fixed biopsy guide attachment and a 17- or 18-gauge guide needle are used.[4-8] First, by reducing the size of the sampling device, one would expect to observe beneficial effects on the complication rate. A Canadian study on amniocentesis demonstrated a significantly lower incidence of fetal loss and maternal complications when a 20-gauge needle was used rather than a larger one.[9] Second, although a transabdominal freehand sampling method may be more difficult to learn than the needle-guided method and certainly requires coordinated teamwork,[10, 11] it allows an easier adjustment of the needle direction when necessitated by involuntary abdominal wall or uterine displacements.

In our experience with first-trimester fetal diagnosis, transabdominal sampling was primarily introduced as a technique to complement transcervical aspiration ideally to achieve a higher sampling success rate, reduce the need for a second transcervical insertion, and reduce complications. For the most part, these objectives seem to have been attained. In the second part of our transabdominal experience, having achieved adequate skill by combining transcervical and transabdominal aspiration, we obtained a sampling success rate of virtually 100% in a variety of anatomic and clinical conditions.[12] A special advantage of the combined use of the two techniques should be evident in cases of multiple pregnancy, where placentas can be reliably sampled independent of their anatomic relationship.

In conclusion, although in our hands transcervical aspiration was confirmed to be the method of choice[12] in clinical practice, the benefit of the transabdominal approach for optimizing the sampling success rate and reducing procedural difficulties was clearly demonstrated. Moreover, the transabdominal method described has the same advantages that have been previously reported.[4-8] In addition, the thinner needle and freehand ultrasound guidance of the sampling system make it easier to perform with a potentially lower risk for maternal and fetal complications.

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REFERENCES

1. Brambati B, Oldrini JA. Methods of chorionic villus sampling. In: Brambati B, Simoni G, Fabro S, eds. Chorionic villus sampling. New York: Marcel Dekker, 1986:73-98.
2. Dumez Y, Goossens M, Boué J, Poenaru L, Dommergues M, Henrion R. Chorionic villi sampling using rigid forceps under ultrasound control. In: Fraccaro M, Simoni G, Brambati B, eds. First trimester fetal diagnosis. New York: Springer-Verlag, 1985:38-45.
3. Gustavii B. Direct vision technique for chorionic villi sampling in 100 diagnostic cases. In: Fraccaro M, Simoni G, Brambati B, eds. First trimester fetal diagnosis. New York: Springer-Verlag, 1985:46-50.
4. Smidt-Jensen S, Hahnemann N, Jensen PKA, Therkelsen AJ. Transabdominal chorionic villi sampling for first trimester fetal diagnosis. In: Fraccaro M, Simoni G, Brambati B, eds. First trimester fetal diagnosis. New York: Springer-Verlag, 1985:51-3.
5. Jackson L. Chorion villus sampling newsletter, January 21, 1986.
6. Simoni G, Terzoli GL, Romitti L. Fetal karyotyping by direct chromosome preparation. In: Brambati B, Sim-

oni G, Fabro S, eds. Chorionic villus sampling. New York: Marcel Dekker, 1986:99-118.

7. Bossi A, Caccamo ML, De Scrilli AM, Milani S. Weight of the Italian newborn infants (from the 32nd to the 43rd week of gestation). Ital J Ped 1980;6:153-70.
8. Maxwell D, Lilford R, Czepulkowski B, Heaton D, Coleman D. Transabdominal chorionic villus sampling. Lancet 1986; i:123-6.
9. Simpson NE, Dallaire L, Miller JR. Prenatal diagnosis of genetic disease in Canada: report of a collaborative study. Can Med Assoc J 1976;115:739-46.
10. Daffos F, Cappella-Pavlovsky M, Forestier F. A new procedure for fetal blood sampling in utero: preliminary results of fifty-three cases. AM J OBSTET GYNECOL 1983; 146:985-7.
11. Hobbins JC, Grannum PA, Romero R, Reece EA, Mahoney MJ. Percutaneous umbilical blood sampling. AM J OBSTET GYNECOL 1985;152:1-6.
12. Brambati B, Oldrini A, Ferrazzi E, Lanzani A. Chorionic villus sampling: an analysis of the obstetric experience of 1,000 cases. Prenat Diagn 1987;7:157-69.

The immediate effects of chorionic villus sampling on fetal movements

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The immediate effects of chorionic villus sampling on the fetus was studied by real-time ultrasound observation of fetal movements. Thirty-minute recordings, directly before and after chorionic villus sampling, were made in 10 women. No changes were observed in the incidence or distribution of the four movement patterns studied. This held true even in the two cases that ended in a spontaneous abortion. It is concluded that chorionic villus sampling does not stimulate movements or alter the intrauterine situation in such a way that fetal movements decrease. (AM J OBSTET GYNECOL 1987;157:137-9.)

Key words: Chorionic villus sampling, real-time ultrasound, fetal movements, first trimester of pregnancy

Chorionic villus sampling has become an increasingly popular technique for early prenatal diagnosis. More than 10,000 investigations have been performed worldwide to date.[1] So far the fetal consequences of this procedure have been assessed in terms of the abortion rate, fetal growth as observed via serial ultrasound, and perinatal outcome.[2-4] With real-time ultrasound equipment, distinct patterns of fetal behavior have been described from 7 weeks of gestation onward.[5] We attempted to determine the acute effects of this invasive diagnostic procedure on fetal motor behavior by means of two 30-minute observations taken immediately before and after chorionic villus sampling.

Patients and methods

Ten multiparous women were selected at random from those referred for chorionic villus sampling. After receiving a detailed explanation of the investigation, they agreed to take part in the study.

Continuous real-time ultrasound images were obtained and recorded on videotape for 30 minutes immediately before and 30 minutes directly after chorionic villus sampling with an Aloka SSD-256, which was equipped with a 3.5 MHz linear array transducer. The fetuses were usually visualized in the mid- or parasagittal longitudinal section. The analysis of fetal motility was carried out during playback of the video recordings in the presence of an independent observer to minimize bias and reduce interobserver error. The incidence and duration of the different movements were marked on an event recorder (model 7754a, Hewlett-Packard, Palo Alto, Calif.) by a handheld push buttons. General movements, startles, hiccups, and breathing movements were analyzed according to the criteria described by de Vries et al.[5] General movements were expressed as a percentage of the time they were present; the other movements were simply counted. In case the recording time differed slightly from 30 minutes, the incidence of movements was normalized by conversion to 30 minutes. The results were analyzed by the Wilcoxon signed rank test.

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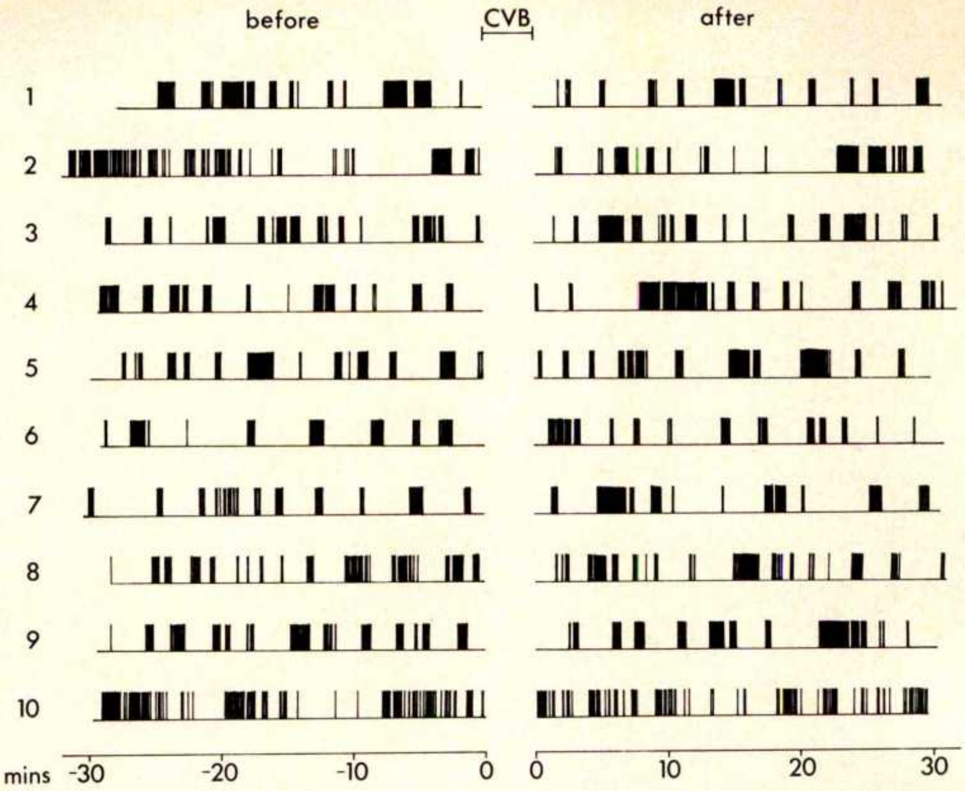

Fig. 1. Compressed actogram of general fetal movements before and after chorionic villus biopsy *(CVB)* in the 10 cases.

Table I. Clinical data on the 10 women, as well as the incidence (percentage of time or number per 30 minutes) of fetal movements before and after chorionic villus sampling

Case No.	*Age (yr)*	*Duration of pregnancy (wk and days)*	*General movements (% of time)*		*Startles (n)*		*Breathing movements (n)*		*Hiccups*	
			Before	*After*	*Before*	*After*	*Before*	*After*	*Before*	*After*
1	37	10	25	18	8	18	24	39	41	51
2	36	11^{+1}	24	27	1	1	0	2	0	1
3	38	9^{+1}	28	25	5	10	0	0	0	2
4	41	9^{+6}	15	20	11	4	13	2	35	11
5	26	10^{+1}	20	17	4	9	4	0	4	11
6	38	9^{+5}	23	24	8	8	1	0	0	1
7	38	9^{+6}	21	24	9	7	2	0	10	10
8	39	9^{+5}	20	22	5	3	0	0	11	21
9	36	9^{+5}	20	19	19	8	10	0	24	33
10	40	11^{+5}	27	19	7	11	22	11	1	9
Mean	37	10^{+1}	22.3	21.4	7.7	7.9	7.6	5.4	12.6	15.0

Chorionic villus sampling was performed with a trophoblast aspiration catheter (Trophocan), which was introduced transcervically under ultrasound guidance. A maximum of three aspirations per session were carried out to obtain approximately 20 mg net weight of good quality villi for analysis. No anesthetic agents or maternal sedation was used. The perineum, vagina, and cervix were thoroughly cleansed with povidine iodine solution before the procedure was begun.

Results

The study population comprised part of the first 100 diagnostic chorionic villus samplings carried out in our department. The overall abortion rate in this series was 3%, with successful sampling in 96 patients and successful karyotyping in 95 patients. Interestingly, two of the three patients who aborted spontaneously after chorionic villus sampling were part of the study population (cases 1 and 10, Table I). In the latter case the gestational sac was accidentally ruptured during the procedure. The results of the procedure were otherwise unremarkable. A mean weight of 36 mg of chorionic villi was obtained after an average of 1.7 aspirations between 9 and 12 weeks of gestation. No abnormal karyotypes were found in the series.

The statistical analyses of fetal movements before and after chorionic villus sampling did not show significant differences for all four modalities analyzed (Table I). In the two cases ending in a spontaneous abortion (7 and 14 days after chorionic villus sampling), no significant change was noted immediately after the procedure, although there was a tendency toward reduced general movements in these fetuses.

The actogram of general fetal movements is displayed in Fig. 1, which shows a high overall incidence of movements both before and after chorionic villus sampling. It also shows that the incidence of general movements directly after chorionic villus sampling was the same as at the end of the second 30-minute recording.

Comment

The only other study on the immediate effects of chorionic villus sampling on the fetus to date was conducted by Löfberg et al.,[6] who describe transient fetal bradycardia during direct-vision sampling by means of the extra-amniotic instillation of 150 ml of physiologic saline. This effect appears to be the result of a rise in intrauterine pressure and consequently is not relevant to our study.

Fetal movements can be observed from 7 weeks' postmenstrual age.[5] There is an early emergence of specific movement patterns; at 16 weeks 15 distinct patterns are already present, which closely resemble those observed in preterm and term newborn infants.[5] When the different movement patterns are rank ordered according to their first appearance, a specific sequence emerges. It has been demonstrated that in embryos and fetuses of type 1 diabetic women, there is a delay of about 1 week in the first occurrence of these movements, except for breathing.[7] Furthermore, it appears that in anencephalic fetuses, movement patterns are (already) qualitatively abnormal in the first half of pregnancy.[8]

In Fig. 1 the compressed actogram of general movements shows that there is already a high base rate of spontaneous fetal movements at 9 to 11 weeks. With respect to these and the other movements studied, the data show that chorionic villus sampling does not stimulate movements or alter the intrauterine situation in such a way as to decrease fetal movements. The latter is known to occur in threatened abortions.[9] In the two cases of a subsequent spontaneous abortion, the incidence of general movements but not the other movements studied was slightly decreased after chorionic villus sampling. However, more data are required to determine the significance of this finding.

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REFERENCES

1. Jackson LG. Chorionic Villus Sampling, Jan. 21, 1986.
2. Gustavii B. Chorion biopsy and miscarriage in first trimester. Lancet 1984;i:562.
3. Brambati B, Simoni S, Danesino C, et al. First trimester fetal diagnosis of genetic disorders: clinical evaluation of 250 cases. J Med Genet 1985;22:92-9.
4. Hogge WA, Shonberg SA, Golbus MS. Prenatal diagnosis by chorionic villus sampling: lessons of the first 600 cases. Prenat Diagn 1985;5:393-400.
5. de Vries JIP, Visser GHA, Prechtl HFR. The emergence of fetal behaviour. 1. Qualitative aspects. Early Hum Dev 1982;7:301-22.
6. Löfberg L, Iosif CS, Edvall H, Gustavii B. Direct-version sampling of chorionic villi during extra-amniotic instillation of physiologic saline solution: effects on intrauterine pressure and fetal heart activity. AM J OBSTET GYNECOL 1985;152:591-2.
7. Visser GHA, Bekedam DJ, Mulder EJH, van Ballegooie E. Delayed emergence of fetal behaviour in type-1-diabetic women. Early Hum Dev 1985;12:167-72.
8. Visser GHA, Laurini RN, de Vries JIP, et al. Abnormal motor behaviour in anencephalic fetuses. Early Hum Dev 1985;12:173-82.
9. Schmidt W, Garoff L, Heberling D, et al. Überwachung der fetalen Bewegungsaktivität mit Real-Time-Ultraschall und deren Bedeutung für den Schwangerschaftsverlauf. Geburtshilfe Frauenheilkd 1981;41:601-6.

Influence of the menstrual cycle on glucose tolerance and insulin secretion

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To evaluate the impact of the menstrual cycle on glucose tolerance and insulin secretion, in the present study we have measured the plasma concentrations of glucose, insulin, and C-peptide during a 2-hour oral glucose tolerance test in 110 healthy, nonobese, regularly menstruating women. Fifty-five women were in the follicular phase, and 55 were in the luteal phase of the cycle. The two groups were well matched for age and body weight. Plasma concentrations of glucose, insulin, and C-peptide either in the fasting state or after the oral glucose load did not differ in the two groups. These results suggest that in nondiabetic women the menstrual cycle has no major effect on glucose tolerance and insulin secretion and that the phase of the menstrual cycle should not be considered in programming and interpreting an oral glucose tolerance test. (AM J OBSTET GYNECOL 1987;157:140-1.)

Key words: Menstrual cycle, oral glucose tolerance test, glucose, insulin, C-peptide

Several hormones, particularly steroids, affect carbohydrate metabolism, and plasma concentration of some of these hormones changes during the menstrual cycle. Nevertheless, in its recommendations about the ways to perform an oral glucose tolerance test, the National Diabetes Data Group[1] did not give instructions about the period of the menstrual cycle in which to carry out an oral glucose tolerance test in fertile women. This probably depends on the discrepancies found in studying the influence of the phases of the menstrual cycle on carbohydrate metabolism.[2-5]

Since the previous studies have been performed in small series of subjects or without considering the confounding effect of obesity, concomitant diseases, drug consumption, and glucose intolerance, we have compared plasma levels of glucose, insulin, and C-peptide during an oral glucose tolerance test in two large groups of nonobese, nondiabetic, healthy women in the follicular or luteal phase of the menstrual cycle.

Material and methods

The study population consisted of 110 nonobese (body mass index < 27 kg/m^2), regularly menstruating women. Fifty-five women were in the follicular phase (days 5 to 10 of the cycle), and 55 were in the luteal phase (days 20 to 25 of the cycle). The two groups were similar in age (37 ± 5 versus 36 ± 5 years) (mean ± SD) and body weight (body mass index of 23 ± 2 versus 23 ± 2 kg/m^2). The women were ambulatory volunteers in good general health with no first-degree relatives with diabetes, negative history of important diseases, normal physical examination, normal blood tests, normal glucose tolerance, and did not take any drug. They underwent a standard 75 gm oral glucose tolerance test, with blood samples at fasting, 1, and 2 hours after glucose ingestion for the determination of plasma glucose (glucose-oxidase enzymatic method) and insulin.[6] In a subset of 44 women, 22 in the follicular phase and 22 in the luteal phase, plasma C-peptide concentrations were also measured.[7] Also these two groups were similar in age (36 ± 4 versus 36 ± 4 years) and body weight (body mass index of 23 ± 2 versus

Table I. Plasma concentrations of glucose, insulin, and C-peptide during a 2-hour oral glucose tolerance test in women in the follicular or luteal phase of the menstrual cycle (mean ± SEM)

	Follicular	*Luteal*
Glucose (mg/dl)		
Fasting	81 ± 1	81 ± 1
1-hr	87 ± 4	86 ± 3
2-hr	77 ± 11	74 ± 9
Insulin (μU/ml)		
Fasting	15 ± 1	13 ± 1
1-hr	66 ± 6	68 ± 8
2-hr	43 ± 3	43 ± 4
C-peptide (pmol/ml)		
Fasting	0.56 ± 0.04	0.62 ± 0.03
1-hr	1.90 ± 0.09	1.97 ± 0.10
2-hr	1.75 ± 0.08	1.64 ± 0.08

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23 ± 2 kg/m^2). Student's t test for unpaired data was used for statistical analysis of the results.

Results

Plasma glucose, insulin, and C-peptide concentrations at fasting and after oral glucose load in women in the follicular or luteal phase of the menstrual cycle are reported in Table I. No significant differences were found in the variables examined.

Comment

Regarding the influence of the menstrual cycle on glucose tolerance, our results are in agreement with those by Spellacy et al.[3] and Cudworth and Veevers[4] as well as with those recently reported by Reid et al.[8] but are in contrast with those by Jarrett and Graver[2] and Peppler et al.[5] However, Jarrett and Graver[2] examined a small series of 10 women in various phases of the menstrual cycle, and Peppler et al.[5] did not take into account the confounding effect of obesity, concomitant diseases, drug consumption, or glucose intolerance. On the other hand, our results are consistent with those by Yki-Jarvinen,[9] who demonstrated that insulin sensitivity does not change during the menstrual cycle, and with those by Pedersen et al.,[10] who showed that in human adipocytes insulin binding to its receptors, basal and insulin-stimulated glucose transport, glucose oxidation, and lipogenesis do not differ in women in the follicular or luteal phase of the menstrual cycle.

Regarding the influence of the menstrual cycle on plasma insulin levels, our results are in agreement with those by Spellacy et al.[3] and Cudworth and Veevers,[4] who measured plasma insulin levels after intravenous and oral glucose load in different phases of the menstrual cycle in 19 and 20 women, respectively. In our study, plasma C-peptide concentrations were also measured during oral glucose tolerance test to confirm that the β-cell secretion rate and not only the peripheral insulin levels do not change during the menstrual cycle. Indeed, there are clinical situations in which plasma insulin and C-peptide levels at fasting and/or after oral glucose load are not unanimously different from normal. This is because of a difference in the metabolism rather than in the secretion of insulin.[11, 12]

In the present study we have included only nonobese woman. Thus, one might hypothesize that the menstrual cycle affects glucose tolerance and/or insulin secretion in obese women. To address this issue, we are now carrying out a large study on obese nondiabetic woman in the follicular or luteal phase of the menstrual cycle. The preliminary results of this study also suggest that in the presence of obesity the menstrual cycle does not significantly affect glucose tolerance and/or insulin secretion.

In conclusion, our results indicate that in nondiabetic women glucose tolerance and insulin secretion do not change during the menstrual cycle, and the phase of the menstrual cycle is not to be considered in programming and interpreting an oral glucose tolerance test. Further studies are needed to elucidate whether the menstrual cycle has a different impact on plasma glucose levels and/or insulin secretion in the presence of impaired glucose tolerance or overt diabetes.

REFERENCES

1. National Diabetes Data Group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 1979;28:1039-57.
2. Jarrett RJ, Graver HJ. Changes in oral glucose tolerance during the menstrual cycle. Br Med J 1968;2:528-9.
3. Spellacy WN, Carlson KL, Schade SL. Menstrual cycle carbohydrate metabolism. AM J OBSTET GYNECOL 1967; 99:382-6.
4. Cudworth AG, Veevers A. Carbohydrate metabolism in the menstrual cycle. Br J Obstet Gynecol 1975;82:162-9.
5. Peppler U, Thefeld W, Wincenty U. Oraler glukosetoleranztest bei frauen in abhangigkeit vom menstruationszyklus. Klin Wochenschr 1978;56:659-69.
6. Hales CN, Randle PJ. Immunoassay of insulin with insulin-antibody precipitate. Biochem J 1963;88:137-46.
7. Kaneko T, Oka H, Munemura M, et al. Radioimmunoassay of human proinsulin C-peptide using synthetic human connecting peptide. Endocrinol Jpn 1974;21:141-5.
8. Reid RL, Greenaway-Coates A, Hahn PM. Oral glucose tolerance during the menstrual cycle in normal women and women with alleged premenstrual "hypoglycemic" attacks: effects of naloxone. J Clin Endocrinol Metab 1986;62:1167-72.
9. Yki-Jarvinen H. Insulin sensitivity during the menstrual cycle. J Clin Endocrinol Metab 1984;59:350-3.
10. Pedersen O, Hjollund E, Lindskov HO. Insulin binding and action on fat cells from young healthy females and males. Am J Physiol 1982;E158-67.
11. Bonora E, Zavaroni I, Coscelli C, Butturini U. Decreased hepatic insulin extraction in subjects with mild glucose intolerance. Metabolism 1983;32:438-44.
12. Bonora E, Zavaroni I, Bruschi F, et al. Peripheral hyperinsulinemia of simple obesity: pancreatic hypersecretion or impaired insulin metabolism? J Clin Endocrinol Metab 1984;59:1121-7.

Proopiomelanocortin-related peptides and methionine enkephalin in human follicular fluid: Changes during the menstrual cycle

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Several studies indicate the presence of different pituitary hormones or neuropeptides in ovarian follicular fluid from various species. Recently our group showed that the ovarian follicular fluid of healthy women contains two of the endogenous opioid peptides, β-endorphin and methionine enkephalin, in concentrations that are tenfold to twentyfold higher than in circulating plasma. The presence of immunoreactive β-lipotropin was also shown. The aim of the present study was to evaluate whether adrenocorticotropic hormone, which in pituitary cells is synthesized from proopiomelanocortin such as β-endorphin and β-lipotropin, is also present in follicular fluid and the possible changes of proopiomelanocortin-related peptides during the menstrual cycle. Concentrations of β-endorphin, methionine enkephalin, adrenocorticotropic hormone, and β-lipotropin were measured in 60 heatlhy menstruating women at different periods of the menstrual cycle (20 during the follicular, 22 in the preovulatory days, and 18 during the luteal phase). Thirteen women participated in an in vitro fertilization program and thus received clomiphene citrate (100 mg/day from the fifth to the ninth day) plus 5000 IU human chorionic gonadotropin before starting the program. All samples were collected at laparoscopy under general anesthesia. In another eight patients fluid was collected from follicular cysts. Peptides were extracted on octadodecasilyl silica columns with 80% methanol in 0.5 mol/L acetic acid. The identity of follicular fluid β-endorphin, methionine enkephalin, adrenocorticotropic hormone, and β-lipotropin and standard peptides was demonstrated with high-pressure liquid chromatography. Peptide concentrations were measured in extracts by radioimmunoassays either directly by (methionine enkephalin and adrenocorticotropic hormone) or after (β-endorphin and β-lipotropin) gel filtration on Sephadex G-75. The concentrations of methionine enkephalin, adrenocorticotropic hormone, and β-lipotropin were similar in the different periods of the cycle. Conversely, β-endorphin concentrations were significantly higher in preovulatory days than in the other periods; no differences were evident between spontaneous and stimulated cycles. These results indicate that proopiomelanocortin-related peptides are present in the follicular fluid and that β-endorphin concentrations change during the menstrual cycle, with the highest values occurring in the preovulatory follicle. (AM J OBSTET GYNECOL 1987;157:142-6.)

Key words: Proopiomelanocortin, methionine enkephalin, β-lipotropin, β-endorphin, menstrual cycle

In the past few years, various peptide hormones have been localized in ovarian tissues and fluids of different species. The follicular fluid of healthy menstruating women has been found to contain large amounts of luteinizing hormone and prolactin, which show significant changes throughout the menstrual cycle.[1] Immunoreactive oxytocin and vasopressin have been identified in the extracts of the human ovary.[2] Immunohistochemical findings from the sheep ovary localize oxytocin in corpora lutea,[3] and in vivo studies indicated that its secretion is stimulated by prostaglandins.[4] Moreover the presence of immunoreactive β-endorphin and immunoreactive adrenocorticotropic hormone have been demonstrated in sheep follicular fluid.[5] Sheep,[5] mouse,[6] and rat[7] ovarian homogenates contain immunoreactive β-endorphin, with cells staining positively for immunoreactive β-endorphin in the corpora lutea[6, 7] or in follicular cells.[6] More recently, our group reported that human follicular fluid contains high amounts of immunoreactive β-endorphin and another endogenous opioid peptide, methionine enkephalin.[8] Both of these opioids are also detectable in the peritoneal fluid, where changes in immunoreactive β-endorphin are related to changes in progesterone concentrations.[8] The presence of immunoreactive

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β-lipotropin has also been shown in eluates from Sephadex G-75 chromatography of follicular and peritoneal fluids.[8]

The aim of the present study was to evaluate the possible presence of immunoreactive adrenocorticotropic hormone in human follicular fluid, since this peptide is synthesized in proopiomelanocortin,[9] the precursor molecule common to β-endorphin and β-lipotropin. The changes in the follicular fluid concentrations of immunoreactive β-endorphin, immunoreactive methionine enkephalin, immunoreactive adrenocorticotropic hormone, and immunoreactive β-lipotropin were measured in the spontaneous menstrual cycle. Moreover, we evaluated peptide concentrations in the follicular fluid in conditions of maximally activated ovarian function, that is, in superovulating women.

Material and methods

Subjects. Sixty healthy menstruating women (21 to 35 years old) were recruited for the present study. All patients underwent laparoscopy for tubal sterilization (n = 16), for diagnosis of tubal sterility (n = 31), or for the in vitro fertilization and embryo transfer program (n = 13).

Subjects were selected on the basis of their regular menstrual cycle (28 ± 2 days). Follicular fluids were collected in 20 subjects during the follicular phase, in 22 during the preovulatory days (−2/−1 day before ovulation), and in the remaining 18 subjects during the luteal period. The estradiol to androstenedione ratio was measured in all follicular fluids. It ranged from 0.04 to 1.1 in the follicular phase, from 12 to 56 in the preovulatory period, from 1.4 to 33 in the luteal phase, and from 9.2 to 86 in the superovulated follicles.

The occurrence of ovulation was monitored during the previous and the current cycle by echographic evaluation of the follicular development (twice a day in those participating in the in vitro fertilization and embryo transfer) and the measurement of basal body temperature. Plasma estradiol, progesterone, follicle-stimulating hormone, and luteinizing hormone levels were measured every 3 days (daily in the women undergoing the in vitro fertilization program). No subject received drugs for at least 6 months; patients participating in the in vitro fertilization and embryo transfer program received clomiphene citrate (Clomid) (100 mg/day for 5 days) (Lepetot, Milan, Italy) from the fifth day of the cycle and human chorionic gonadotropin (Profasi hp 5000) (5000 IU, intramuscularly) (Serono, Rome, Italy) was given on the preovulatory day. Four of the 13 patients became pregnant.

Laparoscopy was performed under general anesthesia, and follicular fluid was aspirated from the graafian follicle through a special needle. Immediately after collection of the sample, aprotinin (1000 Kallikrein IU/ml) (Bayer, Leverkusen, Switzerland) was added to the follicular fluid to reduce proteolytic activity; the sample was then centrifuged (3500 rpm × 15 min) and supernatant was stored at $-20°$ C until assays. In eight additional subjects (30 to 39 years), the fluid contained in the asymptomatic follicular cysts (5 to 8 cm diameter) was aspirated and processed as previously reported.

Extraction and chromatography. Each sample was submitted to a peptide extraction procedure by loading on an octadodecasilyl silica column (Sep-Pak, Waters Associates, Milford, Mass.) previously primed with methanol (Carlo Erba, Milan, Italy) and peptone (BDH Chemicals, Poole, England). The peptides were eluted with 80% methanol in 0.5 mol/L acetic acid, dried with a Speed-vac concentrator (Savant, Hicksville, N.Y.), and reconstituted in 0.8 ml of 0.12 mol/L phosphate buffer, pH 7.4. Before measurement of immunoreactive β-endorphin, immunoreactive methionine enkephalin, immunoreactive adrenocorticotropic hormone, and immunoreactive β-lipotropin in each extract, their presence and identity with respective reference peptides were demonstrated by high-pressure liquid chromatography. Aliquots of follicular fluid from 10 different subjects were pooled, extracted, dried, redissolved in 300 μl of the high-pressure liquid chromatography initial eluent, and underwent chromatography. The high-pressure liquid chromatography system (Waters Associates) consisted of two 6000A pumps, a UK6 injector valve, a 660 gradient programmer, and a 440 ultraviolet detector set at 254 nm. A μBondapak C-18 column was eluted from 75:25 to 60:40 of 0.01 mol/L of hydrochloric acid/acetonitrile over a 15-minute period at a 11.5 ml/min flow rate. Fractions were collected every 15 seconds and checked for the presence of the immunoreactive peptides.

Radioimmunoassays. The concentrations of adrenocorticotropic hormone and methionine enkephalin in each single sample were measured by direct radioimmunoassay of the extracts, whereas levels of β-endorphin and β-lipotropin were determined after the chromatographic separation on a Sephadex G-75 column (45 × 1.5 cm) to avoid cross-reactions in the radioimmunoassay procedures.[8] All measurements were made in duplicate at two different dilutions.

β-Endorphin. Synthetic β-endorphin (Organon, Oss, Netherlands) (β-lipotropin 61-91) was used to prepare the standard curve and as tracer after iodine 125 labeling (chloramine T method). The anti—C-terminal β-endorphin serum (supplied by Professor C. H. Li, San Francisco, Calif.) was used at a final dilution of 1:30,000. It fully recognized human β-endorphin, cross reacted 97% with human β-lipotropin, and 2% with α-endorphin and γ-endorphin. No cross-reactivity occurred with methionine enkephalin, adrenocorticotropic hormone, leucine enkephalin, or β-lipotropin. The incubation procedures are reported elsewhere.[8] The sensitivity of the radioimmunoassay was 1.5 fmol/tube; the intra-assay coefficient of variation was 7.3% ± 1.5%.

Methionine enkephalin. Synthetic methionine enkephalin and anti–methionine enkephalin serum were provided by UCB Bioproducts SA (Brussels, Belgium). The standard peptide was labeled with iodine 125 by the Iodogen method. The antiserum (used at a final dilution of 1:25,000) did not cross react with leucine enkephalin, methionine enkephalin arginine phenylalanine, methionine enkephalin arginine glycine leucine, human or camel β-endorphin, β- or α-lipotropin, α-melanocyte–stimulating hormone, or adrenocorticotropic hormone in a molar excess. The detailed procedures were previously reported.[8] Sensitivity of the radioimmunoassay was 5 fmol/tube; intra-assay coefficient of variation was 5.5% ± 1.0%.

Adrenocorticotropic hormone. All materials were provided by the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases (Bethesda, Md.). Synthetic adrenocorticotropic hormone was labeled with iodine 125 by the chloramine T method. The antiserum (West) was raised against porcine adrenocorticotropic hormone and did not recognizes β- or α-lipotropin, β-endorphin, or methionine enkephalin. It was used at the final dilution of 1:18,000. The details are reported elsewhere.[10] The assay sensitivity was 2.0 fmol/tube; the intra-assay coefficient of variation was 8.2% ± 1.3%.

β-Lipotropin. Human β-lipotropin and the anti-human β-lipotropin serum were kindly provided by Professor C. H. Li. The standard peptide was labeled by use of the chloramine T method. The antiserum recognized the N-terminal portion of the β-lipotropin and cross reacted fully with γ-lipotropin while it did not recognize β-endorphin, adrenocorticotropic hormone, α-melanocyte–stimulating hormone, leucine enkephalin, or methionine enkephalin. Details on the procedures of the radioimmunoassay were previously reported.[8] The assay sensitivity was 1.0 fmol/tube; the intra-assay coefficient of variation was 7.5% ± 1.0%.

Statistical analysis. All the results were corrected for the recovery loss and expressed as femtomoles per milliliter. The statistical comparison among the groups of the patients was performed by analysis of the variance followed by the Duncan test for multiple comparison.

Results

The presence of immunoreactive β-endorphin, immunoreactive methionine enkephalin, immunoreactive adrenocorticotropic hormone, and immunoreactive β-lipotropin and their identity with reference molecules were demonstrated in the follicular fluid extracts through high-pressure liquid chromatography. Immunoreactive peptides elute with the same retention time as reference ones. The elution profiles of immunoreactive β-endorphin and immunoreactive β-lipotropin were not different among the samples of follicular fluid collected at the various periods of the menstrual cycle.

Concentrations of immunoreactive β-endorphin in the follicular fluid collected on the preovulatory days (405 ± 167 fmol/ml, mean ± SE; n = 9) were significantly higher than levels found in fluid collected during the follicular (61.3 ± 48.6; n = 20) and the luteal (83.9 ± 47.5; n = 18) phases of the menstrual cycle (Fig. 1). β-Endorphin concentrations in the follicular fluid of superovulating patients (447 ± 39; n = 13) were higher than those observed in patients during the follicular or luteal phases while falling in a similar range to those measured in preovulatory follicles (Fig. 1). Concentrations of immunoreactive methionine enkephalin, adrenocorticotropic hormone, and β-lipotropin (Fig. 1) did not show significant differences throughout the menstrual cycle. No correlations were found between hormone concentrations and successful fertilization in the subject undergoing the in vitro fertilization program.

Comment

This study confirms the presence of β-endorphin, methionine enkephalin, and β-lipotropin in human follicular fluid[8] and shows that adrenocorticotropic hormone also is detectable in this compartment. Interestingly, high concentrations of proopiomelanocortin-related peptides were found in the human ovarian follicular fluid, which is in agreement with data obtained in sheep.[5] During the preovulatory days β-endorphin levels are higher than in samples collected during the follicular and luteal periods. However, these changes are not accompanied by a similar pattern of β-lipotropin and adrenocorticotropic hormone, whose levels remain constant throughout the menstrual cycle.

Circulating proopiomelanocortin-related peptides are released in a concomitant fashion in basal conditions and after physiologic or pharmacologic stimuli.[11] The differences found in their follicular fluid changes through the menstrual cycle could imply a different proopiomelanocortin processing within the ovary. However, the possibility cannot be excluded that differences between β-endorphin, adrenocorticotropic hormone, and β-lipotropin concentrations in the follicular fluid originate as a result of different proteolytic activity. In fact, recent evidence indicates that the enzymatic processing of proopiomelanocortin in rat ovaries may be more extensive than in the pituitary gland, giving rise to the highest contents of β-endorphin.[7] The positive gradient existing between follicular and plasma

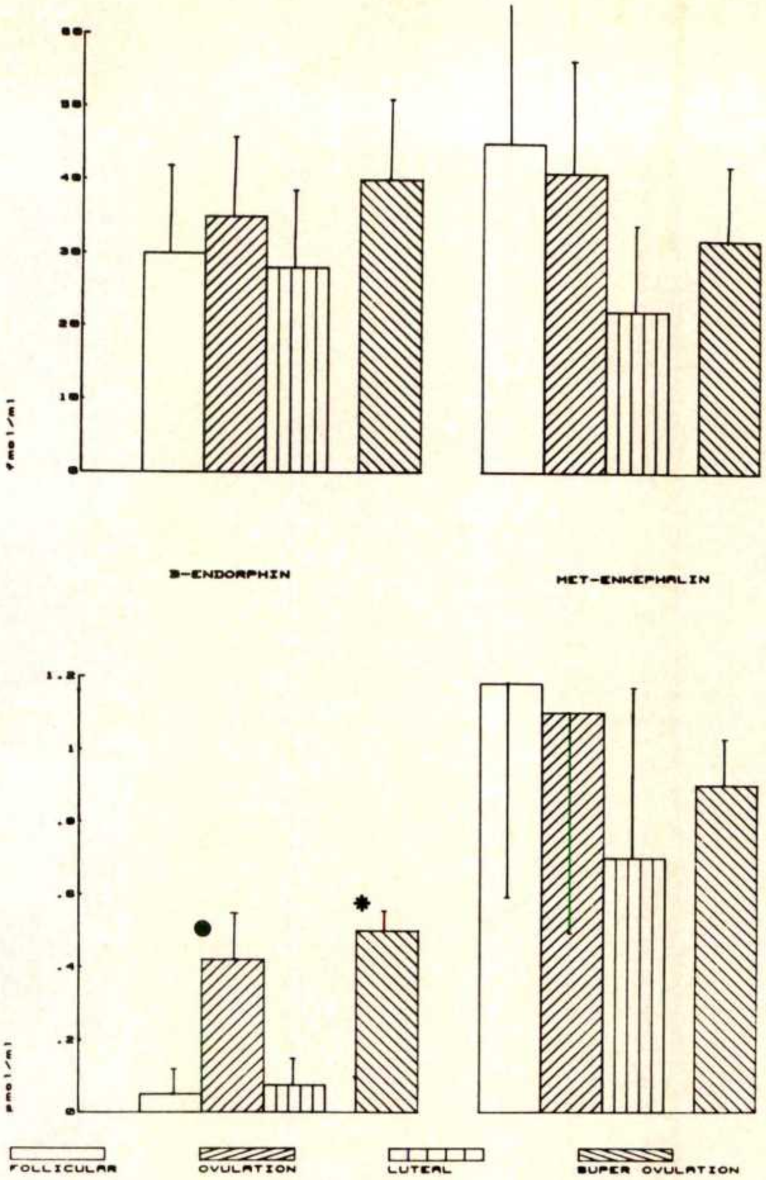

Fig. 1. Follicular fluid concentrations (mean ± standard error) of the four peptides in the different phases of the normal menstrual cycle (follicular, $n = 20$; preovulatory, $n = 9$; and luteal, $n = 18$) and in preovulatory follicles obtained from 13 superovulating patients. (*Asterisk* = $p < 0.01$ versus follicular and luteal phases.)

concentrations suggests the local production of the peptides found herein. Indeed, many reports indicate that both testes[12] and ovaries[13] contain and could synthesize proopiomelanocortin-related peptides and methionine enkephalin. Recently, cells positively staining for β-endorphin have been described in follicles and corpora lutea of human ovaries.[14] The expression of the proopiomelanocortin gene in rat granulosa cells and the increase of ovarian messenger ribonucleic acid after gonadotropin treatment are further evidence that proopiomelanocortin-related peptides may be involved in gonadal function.[13] The present finding that β-endorphin concentrations in spontaneously or pharmacologically induced preovulatory follicles are higher than in other phases of the cycle suggests a linkage between ovulation and ovarian opioids. Accordingly, rat[7] and sheep[5] follicles contain the highest peptide concentration at estrus. However, the biologic significance of ovarian β-endorphin changes still requires clarification, since no correlation was found between follicular β-endorphin and successful in vitro fertilization and embryo transfer.

Other reports also suggest that methionine enkephalin and adrenocorticotropic hormone may play a role in modulating ovarian steroid secretion. Nanomolar concentrations of methionine enkephalin enhance the follicle-stimulating hormone–dependent progesterone secretion from cultured granulosa cells,[15] while adrenocorticotropic hormone inhibits rat ovarian steroidogenesis.[16]

In conclusion, these data first report the presence of adrenocorticotropic hormone in human follicular fluid and indicate that among proopiomelanocortin-related peptides, only β-endorphin shows relevant changes during the menstrual cycle.

REFERENCES

1. McNatty KP, Hunter WM, McNelly AS, Sawers RS. Changes in the concentration of pituitary and steroid hormones in the follicular fluid of human graafian follicles throughout the menstrual cycle J Endocrinol 1975;64:555.
2. Wathes DC, Pickering BT, Swann RW, Porter DG, Hull MCR, Drife JO. Neurohypophysial hormones in the human ovary. Lancet 1982;ii:410.
3. Wathes DC, Swann RW. Is oxytocin an ovarian hormone? Nature 1982;297:225.
4. Flint APF, Sheldrick EL. Ovarian secretion of oxytocin is stimulated by prostaglandin. Nature 1982;297:587.
5. Lim AT, Lolait S, Barlow JW, et al. Immunoreactive β-endorphin in sheep ovary. Nature 1983;303:709.
6. Shaha B,, Margioris A, Liotta AS, Krieger DT, Bardin CW. Demonstration of immunoreactive β-endorphin and α-melanocyte stimulating hormone-related peptides in the ovaries of neonatal, cyclic and pregnant mice. Endocrinology 1984;115:378.
7. Lolait SJ, Auteliano DJ, Lim ATW, Smith AI, Toh BH, Funder JW. Ovarian immunoreactive –endorphin and estrous cycle in the rat. Endocrinology 1985;117:161.
8. Petraglia F, Segre A, Facchinetti F, Campanini D, Ruspa M, Genazzani AR. β-Endorphin and met-enkephalin in peritoneal and ovarian follicular fluids of fertile and postmenopausal women. Fertil Steril 1985;44:615.
9. Mains RE, Eipper B, Ling N. Common precursor to corticotropin and endorphin. Proc Natl Acad Sci USA 1977;74:3014.
10. Facchinetti F, Petraglia F, Nappi G, et al. Different patterns of central and peripheral B-EP, B-LPH and ACTH throughout life. Peptides 1983;4:469.
11. Krieger DT, Liotta AS, Brownstein MJ, Zimmermann EA. ACTH, β-lipotropin and related peptides in brain, pituitary and blood. Recent Prog Horm Res 1980;36:377.
12. Tsong SD, Phillips D, Halmi N, et al. ACTH and β-endorphin-related peptides are present in multiple sites in the reproductive tract of the male rat. Endocrinology 1982;110:2204.
13. Chen CLC, Chang CC. Expression and regulation of proopiomelanocortin (POMC) gene in the ovary and placenta. [Abstract 515]. Baltimore: The Endocrine Society, 1985:129.
14. Aleem FA, Omar RA, ElTabbakh GH. Immunoreactive β-endorphin in human ovary. Fertil Steril 1986;45:507.
15. Facchinetti F, Ruspa M, Turci A, et al. Met-enkephalin enhances FSH-dependent progesterone production from cultured granulosa cells. J Clin Endocrinol Metab 1986;63:1222.
16. Asakai R, Kumai A, Sassa S, Okamoto R. Different effects of two synthetic ACTHs on steroidogenesis and cyclic AMP in isolated luteal cells from rat ovaries [Abstract 3]. In: Proceedings of the International Congress of Endocrinology, Quebec City, Quebec, Canada, July 1-4, 1984;262.

Acute abdomen caused by placenta percreta in the second trimester

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A woman with a history of "in utero" diethylstilbestrol exposure but no prior uterine surgery presented with an acute abdomen at 21 weeks' gestation. She had hemoperitoneum associated with placenta percreta. (AM J OBSTET GYNECOL 1987;157:146-7.)

Key words: Placenta percreta, second trimester, acute abdomen

The case diagnosis of an acute abdomen is notoriously difficult in pregnancy. When evaluating the pregnant patient, the clinician must add pregnancy-related complications to the usual differential diagnosis of abdominal pain. The following case illustrates an unusual cause of acute abdomen in the second trimester of pregnancy.

Case report

A 32-year-old, gravida 3, para 2, black woman was hospitalized at 21 weeks' gestation because of abdominal pain. Her history included diethylstilbestrol exposure in utero, a right ovarian cystectomy 14 years previously, and two uncomplicated vaginal deliveries at term. She had never had uterine surgery. She was first seen at 9 weeks for a routine prenatal visit. Findings were normal except for a hemoglobin of 11 gm/dl. At

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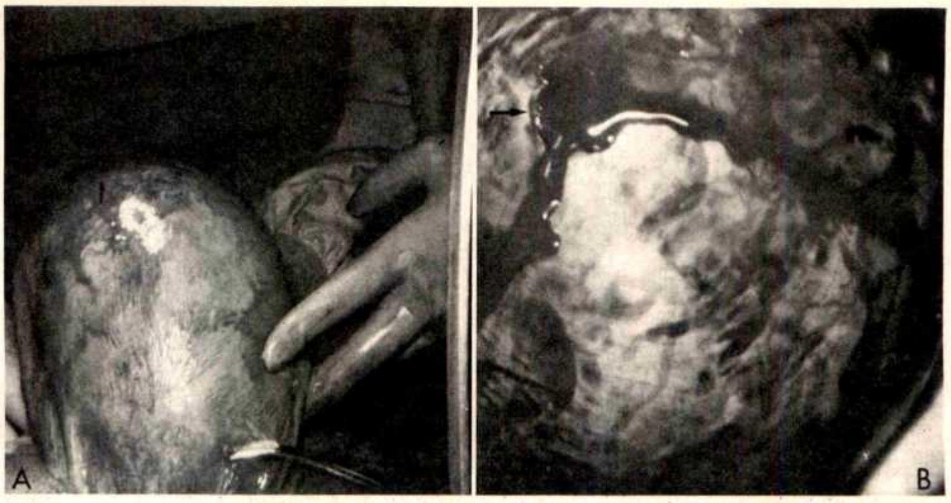

Fig. 1. The uterus as it appeared during operation. *Arrows* mark the bleeding site. In *A*, a prominent vascular pattern is seen at the fundus. A clamp is on the left round ligament. *B* is a close-up of the fundus showing the abnormal vascularity and the bleeding site.

11 weeks she reported fluid leakage; rupture of the membranes could not be detected on examination. At 12 weeks she began spotting. An ultrasound showed a viable pregnancy and confirmed dates. Because a routine maternal serum α-fetoprotein level was elevated, she underwent a second ultrasound with amniocentesis. The amniotic fluid α-fetoprotein and chromosomes were normal (46, XX). The placenta was described as right lateral anterior, and the myometrium was somewhat thicker on the left than on the right.

On the day of admission, the patient awakened at 5 AM with right upper-quadrant pain. She was transferred to our hospital with the provisional diagnosis of viral gastroenteritis or abruptio placentae. On admission at 10 AM, she had stable vital signs. The fetal heart rate was between 150 and 160 bpm. The uterus was diffusely tender, particularly over the right fundus. There was no vaginal bleeding. Mild contractions were occurring every 3 minutes, but the cervix was closed. The hematocrit was 28%; other laboratory tests were normal. She had a resolving upper respiratory infection with a cough.

By 12 PM, the pain was pleuritic and above the uterus. The cervix was unchanged despite continued contractions. A chest x-ray film was negative. An ultrasonogram showed free fluid under the diaphragm, and the myometrium in the fundus was extremely thin. By 6 PM, the patient developed an acute abdomen. Although vital signs were stable, the hematocrit was only 20%. She underwent emergency laparotomy.

The abdomen was filled with blood. The uterine fundus had a reddish purple hue and was traversed by large subserosal vessels. There was slow but persistent oozing from a bleeding site in the midfundus (Fig. 1). We performed a total abdominal hysterectomy. The patient received 7 units of blood during operation. She went home on the fifth postoperative day, and her recovery has been uneventful.

Pathologic examination confirmed a placenta percreta. The cornua were not involved. The female fetus was normal and weighed 350 gm.

Comment

Placenta percreta is an unusual complication of pregnancy, and our case involved an unusual presentation of placenta percreta. Most cases of placenta accreta are diagnosed in the third trimester. There is often an associated uterine factor, such as previous cesarean section (25%), uterine curettage (30%), or manual removal of the placenta (10%), or an associated placental factor, such as placenta previa (34%) or cornual implantation.[1] There was also a disproportionate number of women who are grand multiparas and women over age 35 in Fox's large series.[1] Our patient had none of the above risk factors. We found one similar report in the English literature of a patient with intra-abdominal hemorrhage caused by placenta percreta at roughly the same gestational age.[2] She also had none of the above risk factors except grand multiparity. The association in our case with diethylstilbestrol exposure is intriguing but may be coincidental. We were unable to find any previous reports of an association between placenta percreta and diethylstilbestrol exposure.

In retrospect the diagnosis should not have surprised us. The ultrasound examinations and clinical course were quite consistent with the final diagnosis. However, it was only during operation that we considered placenta percreta. We hope that our case will help others consider this unusual diagnosis more readily.

REFERENCES

1. Fox H. Placenta accreta, 1945-1969. Obstet Gynecol Surv 1972;27:475.
2. Golan A, Ohel G, Menchovsky E, Edelstein T. Haemoperitoneum due to placenta percreta: a case report. S Afr Med J 1977;52:1133.

Prescription drug use before and during pregnancy in a Medicaid population

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This study describes prescription drug use before and during pregnancy and is based on data obtained from the paid Medicaid claims of 18,886 Michigan women aged 15 to 44 years who were delivered of a live infant. Rates of exposure to drugs within 15 therapeutic categories are presented for each of five 90-day periods preceding delivery. Overall dispensed drug use (excluding vitamins) decreased during pregnancy; however, there was still substantial exposure to drugs including drugs that are contraindicated during pregnancy. During the gestational period, study mothers received an average of 3.1 prescriptions for nonvitamin drugs. Black mothers received more exposure to drugs other than vitamins than did white mothers. Black mothers had higher rates of exposure to analgesics, ampicillin, codeine, and vaginal preparations. Similarities between black and white mothers in the use of vitamins with and without other drugs suggest there is no racial difference in attaining prenatal care within the Medicaid system, but that black mothers may have more medical problems that warrant the use of other medications in addition to vitamins during pregnancy. (AM J OBSTET GYNECOL 1987;157:148-56.)

Key words: Drug use; medical assistance, title 19; pregnancy; prenatal care

Current recommendations are that drug use should be avoided by pregnant women and that only those drugs necessary for the health of the mother be given during pregnancy.[1] Any drug prescribed during pregnancy needs to be evaluated for the potential benefits and risks to both mother and fetus. Although numerous studies have been published on drug use in pregnancy in the 1960s and 1970s, little is known about the extent to which drugs are being prescribed for use in pregnancy in the 1980s. Findings from these earlier prospective studies on drug use in pregnancy are summarized in Table I.[2-11] The purpose of this study is to describe drug use in the 15-month period preceding the delivery of a live-born infant in a population of Medicaid recipients with the use of a computerized record system originally created for medical reimbursement purposes. Such a system offers longitudinal data on large numbers of patients in a more cost-efficient manner than do the more traditional prospective study designs. The completeness of drug exposure data in these systems depends on the extent to which drugs are purchased outside the system, the extent to which providers submit billing claims for the drugs, and the use of over-the-counter products that may or may not be captured by the systems. In this study, we have assumed that there is sufficient economic incentive for Medicaid recipients to obtain all their prescriptions within the system and for providers to submit claims for all the drugs dispensed.

Methods

Medicaid claims data. The data presented in this paper are derived from all paid Medicaid claims for Michigan women aged 15 to 44 years (age in 1982) who were delivered of a live infant in a hospital between April 1, 1981, and March 31, 1983. Michigan has a highly developed Medicaid Management Information System in which all health care services covered by Medicaid are recorded in detail. The system contains the basic demographic data of age, race, and sex on each Medicaid recipient. Drugs dispensed by pharmacies and clinics (but not to hospital inpatients) are recorded together with patient identification code, date dispensed, medication strength, and number of units dispensed. Care provided by physicians, hospitals, outpatient clinics, and nursing homes is recorded by patient identification code, provider identification code, date of service, and diagnosis according to the Ninth International Classification of Disease–Clinical Modification scheme.[12]

Study group selection and definitions. Date of birth was defined as the first date of an inpatient diagnosis indicating a live birth. Since the Medicaid data base contains no information regarding when each pregnancy began, such as the date of the last menstrual

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Table I. Review of prospective studies of drug use in pregnancy

Population and time period	*No. of women*	*Duration of follow-up*	*Mean No. of drugs used in pregnancy*
Members of Kaiser Foundation Health Plan, 1960-1961[2]	3,072	3 mo before last menstrual period to birth	3.6*
Patients at Columbia Presbyterian Medical Center, 1953-1957[3]	3,200	First trimester	1.0† (first trimester only)
Patients at Baylor Univ. and Univ. Wisc. affiliates, NS[4]	240	Diagnosis of pregnancy to birth	5.4*
Private obstetric patients, Rochester, New York, NS[5]	67	Last trimester	4.5*
Participants in the Collaborative Perinatal Study, 12 U. S. hospital clinics, 1959-1965[6]	50,282	Diagnosis of pregnancy to birth	3.8
Residents of Malmo, Sweden, 1963-1965[7]	6,376	Diagnosis of pregnancy to birth	5.4
Patients at Univ. Florida, 1974-1976[8]	168	First trimester to birth	11.0*
Tennessee Medicaid recipients, 1975-1976[9]	2,528	15 mo prior to delivery to birth	2.1*†
Patients at Memorial Hosp. Med. Ctr., California, 1973[10]	153	Diagnosis of pregnancy to birth	1.2†
Members of Group Cooperative health plan, 1977-1979[11]	6,837	9 mo preceding live birth	Not calculable

*Includes vitamins and minerals.
†Calculated from data presented.

period, the date when pregnancy began could be estimated only by counting back from the date of birth. For this estimation, a gestational length of 270 days was used. Drug exposures are presented in terms of three 90-day trimesters of pregnancy and two 90-day periods of "prepregnancy."

The study group was limited to women with inpatient delivery codes indicating delivery of a live infant from April 1, 1981, to March 31, 1983, and at least 15 months of available history prior to the delivery. Women were excluded if they were delivered of a stillborn infant (since the length of gestation tends to be shorter in these pregnancies), if they had an induced or spontaneous abortion in the 6-month "prepregnancy" interval (since these would not accurately reflect nonpregnant exposures), and if they had any inpatient Medicaid claim during the 15-month interval before delivery (since the data base does not contain hospital drug exposure data).

Drugs were grouped by therapeutic classes and chemical ingredients were assigned according to the Drug Products Information File (April 1986 version), which allows for grouping into therapeutic classes at the American Hospital Formulary Service Index Therapeutic Category level.[13]

Statistical methods. To test for differences in the distribution of attributes between groups, we used the χ^2 test. To examine the effect of maternal age upon overall (that is, across trimester) drug use by therapeutic drug class, we calculated the χ^2 for linear regression (Armitage trend test) in the proportion of exposures in each 5-year age group on the midpoint of the age group.[14] To adjust for age differences between white and black patients, we used the direct method of adjustment and standardized to the 1982 Michigan vital statistics.

Results

Selection of the study cohort. Of the 57,244 women who were delivered of a live infant during the study period, 28,758 women had a Medicaid claim paid 15 months or more prior to the index birth. Women who had an inpatient hospital admission during the 15 months prior to the index delivery ($n = 8948$) were eliminated from the study group as were women ($n = 924$) who had an induced or spontaneous abortion during the 6 months prior to the index pregnancy. The net result was a study group of 18,886 women who were Medicaid eligible during their pregnancy and the six "prepregnant" months preceding it and for whom we could obtain complete dispensed drug exposure data.

Demographic characteristics of the study group. The demographic characteristics of the study population are shown in Table II. Although there was no difference in the mean maternal age for white and black mothers, there was a significant difference in the 5-year age distribution of white and black mothers ($\chi^2 = 97.0$, $p < 0.0001$). Higher percentages of black mothers than white mothers were 15 to 19 years of age at the time of the index delivery (20.1% versus 15.8%) and were 30 years old and older at the time of the index delivery (14.2% versus 12.0%).

Mothers residing in urban areas, defined as standard

Table II. Age group and racial characteristics of study population (Michigan Medicaid Data, April 1, 1981, to March 31, 1983)

Age group (yr)	*White*		*Black*		*Other*		*Unknown*		*Total*	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%
15-19	1,658	15.8	1,544	20.1	122	20.1	17	12.0	3,341	17.7
20-24	4,949	47.3	3,190	41.6	239	39.4	67	47.5	8,445	44.7
25-29	2,599	24.8	1,849	24.1	142	23.4	35	24.8	4,625	24.5
30-34	943	9.0	807	10.5	71	11.7	14	9.9	1,835	9.7
35-39	262	2.5	231	3.0	27	4.4	5	3.5	525	2.8
40-44	52	0.5	54	0.7	6	1.0	3	2.1	115	0.6
Total	10,463	55.4	7,675	40.6	607	3.2	141	0.7	18,886	100.0

metropolitan statistical areas according to the 1980 United States Bureau of the Census, made up 83.6% of the study population.

Overall drug use. About 80% of the study group (n = 15,094) received at least one nonvitamin prescription during the 15 months preceding the delivery (mean number of prescriptions for those with at least one prescription = 6.7). The remainder received either no Medicaid-reimbursed prescriptions (n = 2238) or only vitamins (n = 1554). There was both an increased likelihood of the use of all drugs other than vitamins with increased maternal age (χ^2 trend = 64.54, $p < 0.001$) and an increased likelihood of the use of vitamins (see the section on vitamin use) with increased maternal age (χ^2 trend = 9.19, $p = 0.001$). Racial differences in overall drug use are discussed in a separate section.

For the 270 days preceding the estimated date of birth, the entire study group received an average of 5.5 prescriptions per woman. If vitamins are excluded, study mothers received an average of 3.1 prescriptions during pregnancy. Among those having prescriptions filled during the gestational period, 10% received 13 or more prescriptions and about 1% received 30 or more prescriptions. Among those women who had at least one prescription, the average number of prescriptions (including vitamins) filled by trimester dropped steadily from 3.9 prescriptions from 450 to 361 days before the estimated date of birth to 3.0 prescriptions in the last trimester of pregnancy. The average number of nonvitamin prescriptions fell from 2.5 to 1.1 in the same time period.

Drug use by trimester and therapeutic category. Fig. 1 shows rates of drug use per 1000 women by "trimester" for each therapeutic category. Not shown because exposure rates were below 2/1000 in any trimester are the antineoplastic agents, the diagnostic agents, enzymes, local anesthetics, oxytocics, and serums, toxoids, and vaccines. The low exposure rates in these categories reflect how the study group was selected and the fact that the data are limited to drugs used in an outpatient environment.

Drug exposures were lower during the estimated 270-day pregnancy period for 10 of the 15 therapeutic classes shown in Fig. 1. Blood formation and coagulation agents (including antianemic drugs such as iron preparations), skin and mucous membrane drugs (including vaginal antifungals and antibiotics), and vitamins were used more frequently during pregnancy. Exposure to gastrointestinal drugs (including antiemetics) also increased, most notably in the first trimester of pregnancy. Use of cardiovascular drugs, which include β-adrenergic receptor blockers but not diuretics, increased in the last trimester of pregnancy and was probably related to treatment of hypertensive disease of pregnancy and cardiac arrhythmias.

Vitamin use. Sixty-nine percent of the mothers received at least one prescription for vitamins during the 270 days preceding the estimated date of birth. Shown in Fig. 1, roughly 3% of the study group had a billing for vitamins in the "prepregnant" period. In the first trimester of pregnancy this figure rose to 33%. By the second trimester of pregnancy more than half of the mothers received at least one prescription for vitamins. The last trimester of pregnancy saw no growth in the rate of vitamin use.

Rates of exposure to the chemical ingredients of dispensed drugs. Since the record system allows for the assignment of specific chemical ingredients for each drug product, it is possible to examine rates of exposure to the chemical ingredients of dispensed drugs (for example, a woman given Darvocet-N 50 would be counted as exposed to both acetaminophen and propoxyphene napsylate). In Table III, these rates (excluding vitamins and minerals) are presented in descending order of their overall rate and for each of the five 90-day periods before the estimated date of birth.

Ampicillin, the most frequent chemical constituent of dispensed drugs (excluding vitamins and minerals) during the entire 15-month period, was used by 26% of the cohort. About one quarter of the women in our study group were given a codeine-containing prescription during the 15-month perion; these women re-

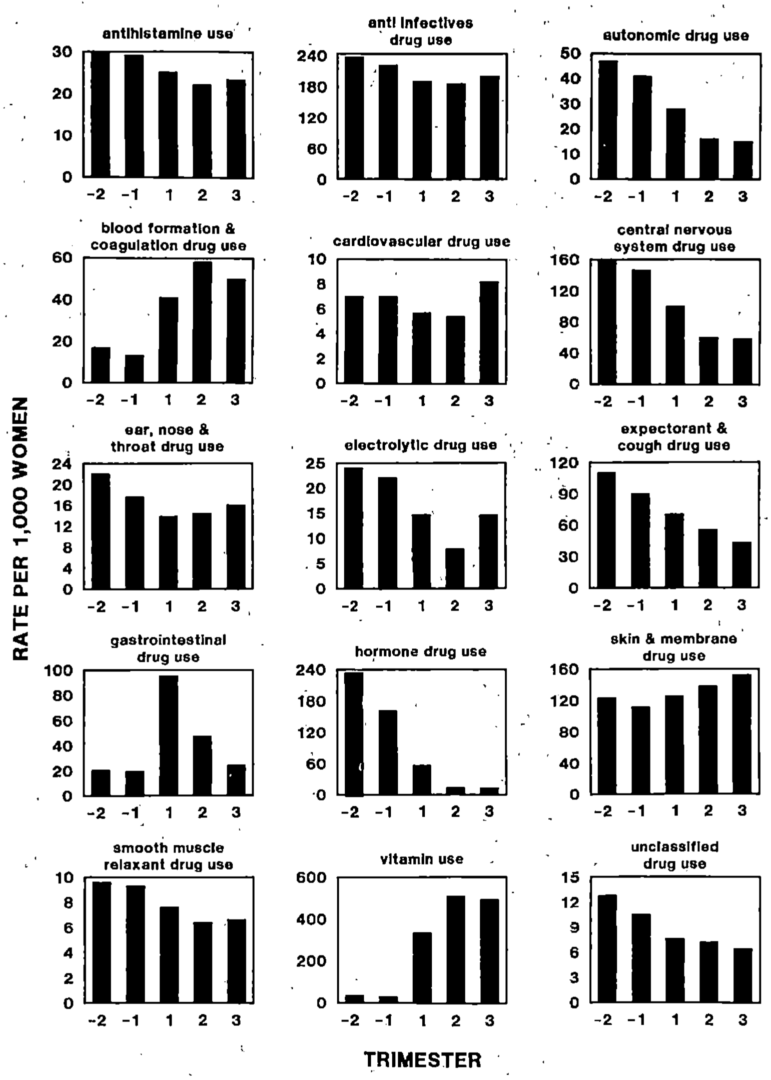

Fig. 1. Rate of drug use per 1000 women for medications in designated therapeutic categories during each of two trimesters prior to the estimated date of conception and the three trimesters of pregnancy. Note that different ordinate scales are used (Michigan Medicaid live delivery data, April 1, 1981, to March 31, 1983).

ceived an average of 2.4 prescriptions for codeine-containing products. Roughly 3% of codeine users had 10 or more prescriptions filled for codeine. Codeine use decreased by more than 50% by the end of pregnancy but was still the fourth most common drug exposure during the last trimester of pregnancy. Codeine is a "hidden" ingredient in many cough and cold preparations, which may partially explain its high ranking. Such wide use in pregnancy is not surprising since codeine is the second most common chemical constituent in dispensed drugs in the general population.[15] Docusate sodium, used by 25% of the women overall, was the most common exposure during the 270 days preceding the estimated date of delivery. Most women were exposed to docusate in vitamin preparations (85%); the remainder were given other docusate-containing products. As would be expected, ingredients found in oral contraceptives showed a dramatic drop as pregnancy was recognized. However, some women apparently continued to have oral contraceptive prescriptions filled during pregnancy. Doxylamine (the active ingredient found in the antinauseant Bendectin) showed the expected peak in the first trimester of pregnancy with gradual tapering as pregnancy progressed. Drugs used to treat vaginal infections increased in use with pregnancy reflecting the well-recognized increase in vaginal yeast infections occurring during pregnancy. The use of hydrocortisone also increased somewhat as pregnancy progressed. Tetracycline and doxycycline (both of which are contraindicated during pregnancy) decreased in use as pregnancy was recognized but they did continue to be used. The urinary antibacterial

Table III. Exposure rates by trimester for chemical constituents of all dispensed drugs (excluding vitamins) for those chemical constituents with an overall usage rate of ≥20/1000 women (Michigan Medicaid data, April 1, 1981, to March 31, 1983)

	Exposure rates per 1000 women by time before estimated date of birth					
Chemical constituent	*Overall*	*450-361 days*	*360-271 days*	*270-181 days*	*180-91 days*	*90-0 days*
Ampicillin	263	62	58	66	77	84
Codeine	256	105	91	68	50	45
Docusate sodium	253	8	7	108	167	158
Acetaminophen	184	74	66	45	31	30
Ethinyl estradiol	171	140	92	27	3	2
Norethindrone	167	138	91	26	3	2
Miconazole nitrate	150	25	22	35	46	57
Erythromycin	148	46	39	31	27	25
Penicillin V	138	40	38	29	27	27
Pseudoephedrine	128	50	41	28	21	15
Aspirin	113	51	43	29	15	11
Doxylamine	112	2	2	81	39	15
Mestranol	103	87	56	14	2	1
Phenylpropanolamine	100	38	30	22	20	15
Aminacrine	87	20	18	22	23	20
Phenylephrine	87	35	25	19	14	10
Phenacetin*	86	40	33	21	11	8
Amoxicillin	85	19	17	19	19	23
Cephalexin	82	23	22	19	15	19
Chlorpheniramine	81	31	25	17	16	12
Sulfanilamide	80	19	17	20	19	17
Promethazine	79	27	21	18	14	11
Diphenhydramine	79	25	24	19	18	17
Metronidazole	77	24	21	11	12	17
Norgestrel	76	62	40	11	1	1
Hydrocortisone	73	20	17	13	16	19
Sulfamethoxazole	69	24	23	15	8	6
Triprolidine	68	22	19	15	13	11
Neomycin	67	21	15	12	12	14
Tetracycline	67	29	28	14	4	2
Clotrimazole	65	14	13	15	14	18
Guaifenesin	63	23	19	13	11	10

*Estimates of phenacetin exposure may be artificially high because of the many product reformations that occurred during the late 1970s and early 1980s.

nitrofurantoin increased in use with pregnancy despite the fact that the manufacturer's package insert (Norwich-Eaton) carries a warning against use of the drug at term because of the risk of hemolytic anemia in the infant.

Racial differences in drug use. For all drugs other than vitamins, black mothers were more likely to have had a drug claim during the 15 months prior to delivery than were white mothers (82% versus 79%, $\chi^2 = 20.94$, $p < 0.001$) or mothers of other racial minorities (82% versus 74%, $\chi^2 = 20.51$, $p < 0.001$).

Differences between white and black mothers in the use of all dispensed drugs other than vitamins were not accounted for by differences in the age distribution of the mothers. After age adjustment, the increased use of all drugs other than vitamins by black women was still evident in each of the five 90-day periods preceding the estimated date of delivery (Fig. 2). The difference between black and white mothers was greatest in the first trimester of pregnancy when 45.4% of the black mothers and 37.6% of the white mothers received a drug other than vitamins. By the last trimester of pregnancy the difference in exposure to drugs other than vitamins had diminished with 41.6% of the black mothers receiving a drug other than vitamins and 39.6% of the white mothers receiving a drug other than vitamins.

After adjustment for age differences, black mothers were less likely than white mothers to have used only vitamins (Fig. 3) in the first two trimesters of pregnancy. However, this difference between black and white mothers disappears when the use of vitamins with or without other drugs is examined (Fig. 4). This seems to indicate that there are no racial differences in the attainment of prenatal care (as indicated by vitamin use) within the Medicaid system but that black mothers may have more medical problems warranting the use of

Table III—Cont'd

Chemical constituent	*Exposure rates per 1000 women by time before estimated date of birth*					
	Overall	*450-361 days*	*360-271 days*	*270-181 days*	*180-91 days*	*90-0 days*
Trimethoprim	58	20	20	13	6	5
Nystatin	52	14	10	11	11	13
Atropine	50	16	15	11	7	6
Caffeine	49	21	19	13	9	6
Ipecac	49	16	13	10	9	7
Brompheniramine	47	17	14	11	8	6
Triamcinolone	44	14	10	10	9	10
Sulfacetamide	44	12	9	10	9	8
Polymyxin B	42	13	10	7	7	8
Phenobarbital	42	16	14	11	6	7
Hyoscyamine	41	14	13	10	5	4
Urea	40	10	9	10	8	7
Propoxyphene	40	19	14	7	4	2
Isopropamide	39	12	11	8	8	7
Lindane	39	10	9	6	8	10
Betamethasone	38	11	10	9	10	10
Guaiacol potassium sulfonate	37	12	10	8	6	5
Gramicidin	36	11	8	7	6	8
Ethynodiol diacetate	33	27	17	4	1	1
Sulfanilylbenzamide	33	8	6	8	7	6
Sulfathiazole	33	8	6	8	7	6
Ibuprofen	33	13	13	7	2	2
Doxycycline	31	12	13	6	2	1
Dexbrompheniramine	30	14	11	6	3	2
Scopolamine	30	11	10	7	3	2
Zomepirac	28	8	12	7	3	2
Phenazopyridine	28	9	9	6	4	3
Hydroxyzine	27	11	10	6	3	3
Butalbital	25	9	9	6	4	2
Theophylline	24	7	6	6	5	5
Hydrochlorothiazide	23	9	8	6	3	7
Orphenadrine citrate	23	9	9	4	2	1
Chlordiazepoxide	23	10	9	6	2	2
Nitrofurantoin	21	3	3	3	6	8
Furosemide	20	9	9	6	3	4
Phenyltoloxamine citrate	20	8	5	4	3	2

other medications in addition to vitamins in pregnancy.

Racial differences in exposure to chemical ingredients of dispensed drugs. Black mothers had 18 different chemical entities with an exposure rate of ≥10% while white mothers had 12. Black women were exposed to more analgesic preparations. Overall 22.3% of black mothers were exposed to acetaminophen, 14.4% were exposed to aspirin, and 11.0% were exposed to phenacetin. In white women, only acetaminophen had an exposure rate >10%. The overall exposure rate for ampicillin was higher in black women (32.1%) than in white women (22.4%) as was the overall exposure rate for codeine (30.8% for black mothers versus 22.3% for white mothers). Black mothers had much higher exposure rates to vaginal preparations than did white mothers. Overall rates of exposure to miconazole, aminacrine, metronidazole, and sulfanilamide were all >10% in black women while in white women they were <10%. Overall exposure rates to docusate sodium were similiar for black and white women (25.1 versus 25.3). White mothers had greater overall exposure rates than black mothers for mestranol (10.9% versus 9.7%), erythromycin (16.0% versus 13.6%), and doxylamine succinate (12.0% versus 10.2%).

Comment

This study looked at drug use in a large group of women both before and during pregnancy to infer what effect, if any, pregnancy had on drug prescribing. Although overall drug use (excluding vitamins) did decrease during pregnancy, there was still a substantial amount of drug exposure, including exposure to drugs such as tetracycline, which is contraindicated during pregnancy. Pregnant women were most frequently exposed to vitamins, antiinfectives, skin and mucous membrane drugs (mainly in vaginitis treatments), and central nervous system drugs (mainly analgesics). Although vitamins were the most commonly used drugs

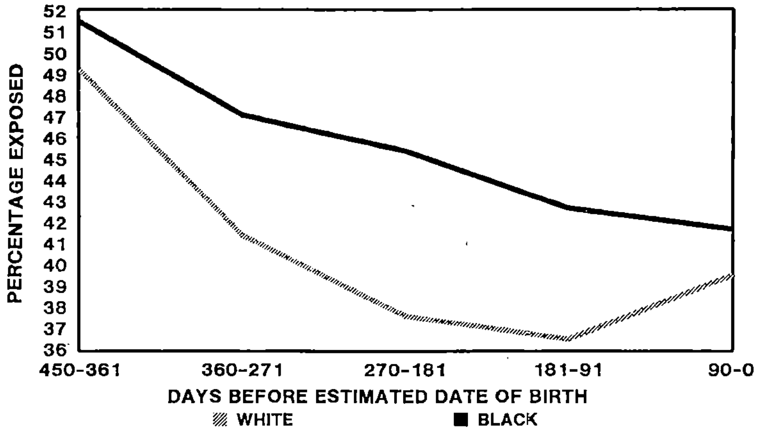

Fig. 2. Racial differences in exposure to prescribed drugs other than vitamins adjusted for maternal age (Michigan Medicaid live delivery data, April 1, 1981, to March 31, 1983).

in pregnancy, current data indicate that a substantial proportion of women did not receive vitamin supplements (roughly 30%) and those who did receive them did not necessarily get them regularly. We cannot rule out the possibility that women may have purchased vitamin supplements outside the Medicaid program, but economic factors would seem to minimize this practice. Drug exposure differed considerably between black and white mothers. Black mothers had a higher exposure rate to drugs other than vitamins and were exposed to a greater number of drug chemicals. Racial differences were not seen for vitamin use.

Several factors must be considered in interpreting the results of this study. The population under study is a fairly homogeneous one consisting of poor, predominantly urban women. Because we required each individual have a billing history dating back at least 15 months prior to the live birth, it is likely that this population is underrepresented by primiparous women. This is because many women become Medicaid eligible only after having a first pregnancy and qualifying for Aid to Families with Dependent Children. Because we required our study group have no hospitalizations in the 15 months prior to the delivery, it is likely that we selected a healthier population than we would otherwise have obtained from Medicaid data.

This study is drawn from a data base generated for medical reimbursement purposes so we are not able to obtain many data elements that would be available in a medical record (for example, cigarette smoking history, parity, or any measurement data such as blood pressure or laboratory values). In this study, in particular, we lack information on the date of the last menstrual period, a critical element in determining the gestational length. Indeed, the information we have, the date on the hospital bill for services connected with the birth and delivery of a live infant, only approximates the actual date of birth. Despite these limitations, we see patterns in the use of drugs (for example, in the vitamin and gastrointestinal therapeutic categories) that are consistent with what one would expect for the three trimesters of pregnancy.

The use of an invariable gestational period of 270 days fails to take into account the fact that gestational length follows a somewhat skewed normal distribution and that a substantial proportion of live births occurs outside of the "usual" 259- to 279-day gestational length. Vital statistics data (1982) for Michigan indicate that 8.9% of all live births for which the date of the last menses is reported have gestational lengths of <259 days and 32.9% have gestational lengths >280 days. Thus a substantial amount of misclassification occurs between the first trimester of pregnancy and the first 90-day period prior to pregnancy. For this reason, we have generally chosen to report drug usage over the five 90-day time periods rather than to dichotomize use into categories during pregnancy and before pregnancy. The reader is cautioned that the best use of these data is for review of trends across the five different time periods.

Despite these caveats, we believe these data are useful because they offer longitudinal prescription drug information on a large number of patients in a cost-efficient manner. These data do not suffer from the bias of patient recall. Because the data are based on prescriptions actually filled, this information is one step closer to what is actually consumed by the patient than what a physician prescribes and records in an outpatient medical chart. Moreover, the computerization of the system has allowed us to link drug products to their actual chemical constituents.

The findings of this study differ in several ways from

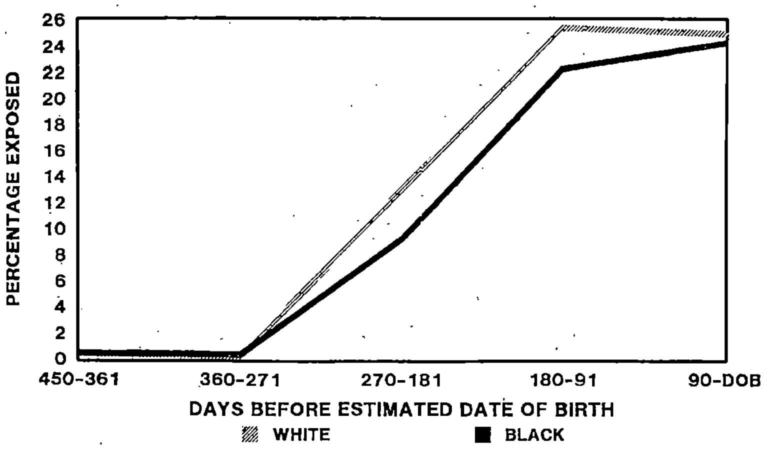

Fig. 3. Racial differences in use of vitamins alone (without any other dispensed medication) adjusted for maternal age (Michigan Medicaid live delivery data, April 1, 1981, to March 31, 1983).

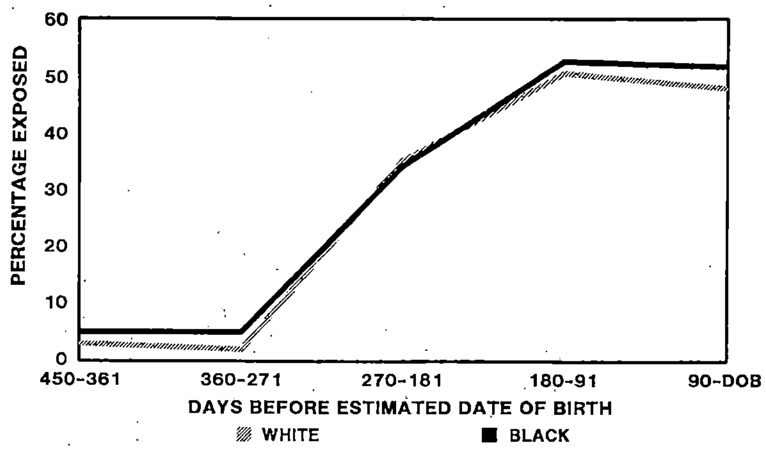

Fig. 4. Racial differences in use of vitamins with or without the use of other medications adjusted for maternal age (Michigan Medicaid live delivery data, April 1, 1981, to March 31, 1983).

other studies regarding drug use in pregnancy (see Table I). In our study, the mean number of drugs per pregnancy was 3.1 excluding vitamins. This is slightly more than the major studies reported in the last decade. The most methodologically analogous study to ours is the one reported by Brocklebank et al.[9] In that study, white women were prescribed more systemic medication than were black women. No mention was made of an adjustment procedure for age differences between white and black mothers; such a procedure may partially explain the differences between studies. Both studies show that pregnant women are exposed to a substantial amount of drugs including drugs that ought to be avoided in pregnancy.

Studies such as this one are important for quantitating the amount of dispensed drug exposure that occurs in pregnancy and giving practitioners some sense of how their own prescribing practices may be viewed. Since most drugs have not been evaluated for safety during pregnancy and lactation, their potential teratogenic or fetocidal effects are unknown. Many drugs slip into use during pregnancy when it is felt that the mother could potentially benefit from the treatment and that the drug has been in the marketplace sufficiently long for a number of nonintentional exposures to have occurred. This type of experience can lull clinicians into a false sense of security especially where infrequently used drugs resulting in rare adverse outcomes such as birth defects are concerned. The relative rarity of individual birth defects makes any epidemiologic study of potential drug-induced etiologies most difficult to study. The situation is one in which neither retrospective nor prospective methodologies are adequate to rule out all but the most elevated risks. Exploration of data bases such as this one hold some hope of providing additional epidemiologic experience, particularly if data on the child's health can be linked to drug exposure in the pregnancy.

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REFERENCES

1. Bennett DR, ed. AMA drug evaluations. Chicago: American Medical Association, 1983.
2. Peckham CH, King RW. A study of intercurrent conditions observed during pregnancy. AM J OBSTET GYNECOL 1963;87:609.
3. Mellin GW. Drugs in the first trimester of pregnancy and the fetal life of *Homo sapiens*. AM J OBSTET GYNECOL 1964;90:1169.
4. Nora JJ, Nora AH, Sommerville RJ, et al. Maternal exposure to potential teratogens. JAMA 1967;202:91.
5. Bleyer WA, Au WY, Lange WA, et al. Studies on the detection of ADRs in the newborn. JAMA 1970;213:2046.
6. Slone D, Heinonen OP, Monson RR, et al. Maternal drug exposure fetal abnormalities. Clin Pharmacol Ther 1973;14:648.
7. Kullander S, Kallen B. A prospective study of drugs and pregnancy. Acta Obstet Gynecol Scand 1976;55:25.
8. Doering PL, Stewart RB. The extent and character of drug consumption during pregnancy. JAMA 1978;239:843.
9. Brocklebank JC, Ray WA, Federspiel CF, et al. Drug prescribing during pregnancy. AM J OBSTET GYNECOL 1978;132:235.
10. Bodendolfer TW, Briggs GG, Gunning JE, et al. Obtaining drug exposure histories during pregnancy. AM J OBSTET GYNECOL 1979;135:490.
11. Jick H, Holmes LB, Hunter JR, et al. First trimester drug use and congenital disorders. JAMA 1981;246:343.
12. World Health Organization. International classification of diseases. 9th rev. Clinical modification. 2d ed. Washington DC: United States Department of Health and Human Services, 1980.
13. Mc Evoy GK, McQuarrie GM, eds. American hospital formulary service: drug information 85. Bethesda: American Society of Hospital Pharmacists, 1985.
14. Armitage P. Tests for linear trends in proportions and frequencies. Biometrics 1955;11:375.
15. Baum C, Kennedy DL, Forbes MB, et al. Drug use in the United States in 1981. JAMA 1984;251:1293.

The effect of oxytocin infusion on the pharmacokinetics of intramuscular magnesium sulfate therapy

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The effect of oxytocin infusion on the pharmacokinetics of standard intramuscular magnesium sulfate therapy was determined in 18 women with preeclampsia; the results were compared with those in seven women with preeclampsia who did not receive oxytocin. Oxytocin had no significant effects on the maternal serum magnesium and calcium ion concentrations, nor did oxytocin appear to affect the magnesium or calcium concentrations in fetal umbilical cord blood. Urinary excretion of magnesium rose 21-fold and calcium excretion rose threefold in patients receiving intramuscular magnesium sulfate in both the oxytocin and the nonoxytocin groups. Sixty-five percent of the administered magnesium was excreted during the treatment period, again with no significant differences between the oxytocin and the nonoxytocin groups. These results indicate that oxytocin does not affect the pharmacokinetics of intramuscular magnesium sulfate and no dosage adjustment of magnesium sulfate is required when oxytocin is used to induce or augment labor or when it is given during the postpartum period. (AM J OBSTET GYNECOL 1987;157:156-60.)

Key words: Magnesium sulfate, oxytocin, preeclampsia

Magnesium sulfate and oxytocin are frequently used as therapeutic agents in the management of preeclampsia. Although the metabolism of magnesium sulfate has been previously described,[1-10] the effect of oxytocin on that metabolism is less certain. Oxytocin, with its antidiuretic effect, could potentially affect the renal excretion of magnesium. The purpose of this study was to

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Table I. Comparison of maternal serum and urine levels (mean ± SEM) of magnesium and calcium after magnesium sulfate treatment in 18 women who received oxytocin versus seven women not requiring oxytocin for labor

	Baseline	*Average treatment value*	*Change from baseline*
Serum ionized magnesium (mg/dl)			
Oxytocin	1.66 ± 0.04	4.71 ± 0.28	284%
No oxytocin	1.50 ± 0.12	4.24 ± 0.32	284%
Serum ionized calcium (mg/dl)			
Oxytocin	9.16 ± 0.15	8.63 ± 0.14	−6%
No oxytocin	8.97 ± 0.49	8.50 ± 0.33	−5%
Urinary ionized magnesium (mg/hr)			
Oxytocin	4.33 ± 1.11	88.83 ± 2.86	21×
No oxytocin	4.25 ± 1.11	91.35 ± 2.41	21×
Urinary ionized calcium (mg/hr)			
Oxytocin	3.56 ± 1.11	10.84 ± 0.84	3×
No oxytocin	3.50 ± 2.18	10.71 ± 0.66	3×

Table II. Maternal-fetal magnesium and calcium relationships

	Trough maternal serum (mg/dl)	*Umbilical artery (mg/dl)*	*Umbilical vein (mg/dl)*	*Ratio of cord blood to maternal serum*
Magnesium				
Control group (n = 7)	1.60 ± 0.12	1.49 ± 0.18	1.50 ± 0.18	0.94
Magnesium sulfate				
No oxytocin (n = 7)	4.24 ± 0.32	3.80 ± 0.34	3.90 ± 0.33	0.90
Oxytocin (n = 18)	4.71 ± 0.28	4.20 ± 0.24	4.10 ± 0.23	0.89
Calcium				
Control group (n = 7)	8.96 ± 0.42	11.21 ± 0.19	11.19 ± 0.20	1.25
Magnesium sulfate				
No oxytocin (n = 7)	8.50 ± 0.33	10.65 ± 0.28	10.65 ± 0.18	1.25
Oxytocin (n = 18)	8.63 ± 0.14	10.96 ± 0.21	10.89 ± 0.19	1.26

further delineate the pharmacokinetics of standard intramuscular magnesium sulfate therapy with and without concomitant oxytocin infusion.

Material and methods

Twenty-five women with preeclampsia who were treated with intramuscular magnesium sulfate at Wilford Hall United States Air Force Medical Center were included in this study. A standard magnesium sulfate regimen that consisted of a loading dose of 4 gm intravenously and 10 gm intramuscularly was used. This was followed by maintenance therapy of 5 gm intramuscularly every 4 hours during labor and for 24 hours post partum.[11] Eighteen of these 25 women also received an intravenous oxytocin infusion during the intrapartum period for either induction or augmentation of labor and all women received oxytocin as a uterotonic agent for 4 to 6 hours post partum.

Prior to initiation of magnesium sulfate therapy, baseline levels of magnesium and calcium were measured in maternal serum and urine. During treatment serum magnesium and calcium levels were measured every 4 hours (trough levels), and urinary levels of magnesium and calcium were determined hourly. In addition, magnesium and calcium levels were measured in arterial and venous cord blood collected at delivery.

The urinary magnesium and calcium levels were determined by atomic absorption spectrophotometry. Maternal and cord blood levels of magnesium and calcium were determined with the Dupont Automated Clinical Analyzer, which employs a colorimetric reaction to determine the ion concentration.

Statistical analysis of data collected was performed with the Mann-Whitney and Wilcoxon signed rank sum tests where appropriate.

Results

Maternal serum levels. Serum magnesium levels rose by 284% during intramuscular magnesium sulfate therapy in women who did and did not require oxytocin for augmentation or induction of labor (Table I, Fig. 1). Serum calcium levels similarly decreased during magnesium sulfate therapy in both the oxytocin and the nonoxytocin groups (Table I).

Urinary magnesium. Baseline magnesium excretion rates were similar (p = NS) in the oxytocin and nonoxytocin groups (Table I). After initiation of magnesium sulfate therapy, urinary excretion of magnesium increased steadily in both groups (oxytocin and non-

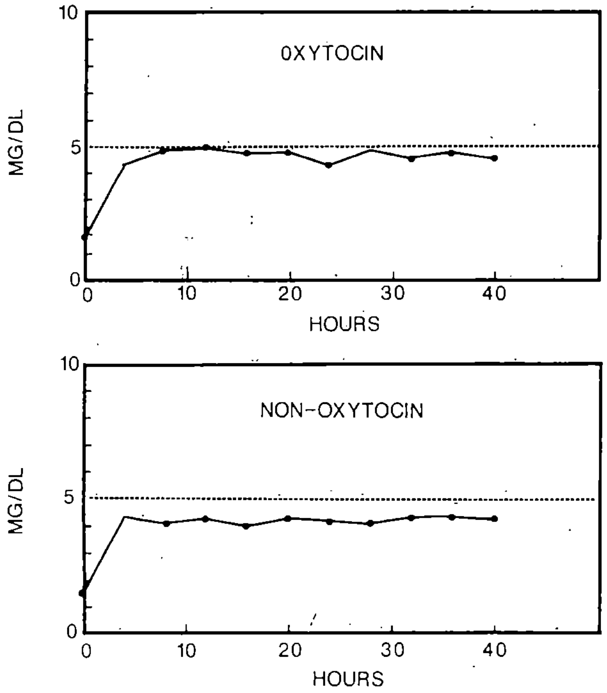

Fig. 1. Serum magnesium levels in 18 women treated with magnesium sulfate who received oxytocin for labor induction or augmentation as compared with seven women who received only magnesium sulfate.

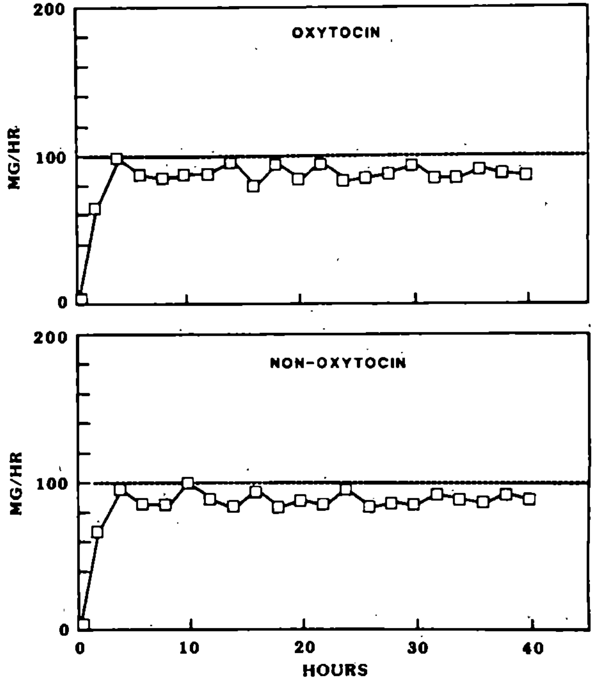

Fig. 2. Urinary magnesium excretion rates in 18 women treated with magnesium sulfate who received oxytocin for induction or augmentation of labor. These women were compared with seven who were treated with magnesium sulfate but did not require oxytocin during labor.

oxytocin) for the first 4 hours of therapy, then plateaued at 88.8 ± 2.86 and 91.36 ± 2.41 mg/hr, respectively (Fig. 2). Urinary excretion of magnesium was not affected by oxytocin infusion either during or after delivery. The mean magnesium excretion rate during oxytocin infusion was 91.63 ± 4.52 mg/hr versus 91.05 ± 6.10 mg/hr in these same patients after oxytocin was discontinued in the postpartum period. The increases in urinary magnesium excretion during magnesium sulfate therapy represent a 21-fold rise regardless of whether oxytocin was being infused.

During the treatment period, which extended from the initiation of magnesium sulfate therapy until 4 hours after the last intramuscular injection, women who required oxytocin for labor excreted 66% ± 4% of the magnesium administered and those women not requiring oxytocin excreted 64% ± 4% of the administered dose (p = NS). Again no significant difference was seen in magnesium ion metabolism as a result of the addition of an oxytocin infusion. Fig. 3 illustrates the progressive increase in the percent of magnesium administered intramuscularly that is excreted in the maternal urine. There was no significant difference between the oxytocin and the nonoxytocin groups.

Urinary calcium. Mean urinary calcium excretion rates increased threefold during maternal magnesium sulfate therapy irrespective of the concomitant use of intrapartum oxytocin (p = NS, Table I). After initiation of magnesium sulfate therapy, urinary calcium excretion rose steadily for the first 4 hours of treatment and then plateaued at 10.8 ± 0.84 mg/hr in the oxytocin group and 10.7 ± 0.66 mg/hr in the nonoxytocin group (Fig. 4, p = NS).

Cord blood. Umbilical arterial and venous magnesium ion concentrations were similar in women who were receiving magnesium sulfate with and without concomitant oxytocin. The umbilical cord/maternal serum ratios were also similar in both groups of women and their fetuses. Additionally, use of magnesium sulfate with or without oxytocin had no effect on umbilical artery or vein/maternal serum calcium ion concentration ratios (Table II).

Comment

Magnesium administered parenterally is initially distributed into the intravascular compartment. The intravascular unbound ion (75%) diffuses into the extravascular-extracellular space, into bone, and across the placenta and fetal membranes and into the fetus and amniotic fluid. Magnesium is excreted by the maternal kidney, which represents the sole route of elimination.[1, 9, 12] In the kidney, unbound plasma magnesium is filtered by the glomerulus and reabsorbed in the tubules. The fraction of magnesium reabsorbed is

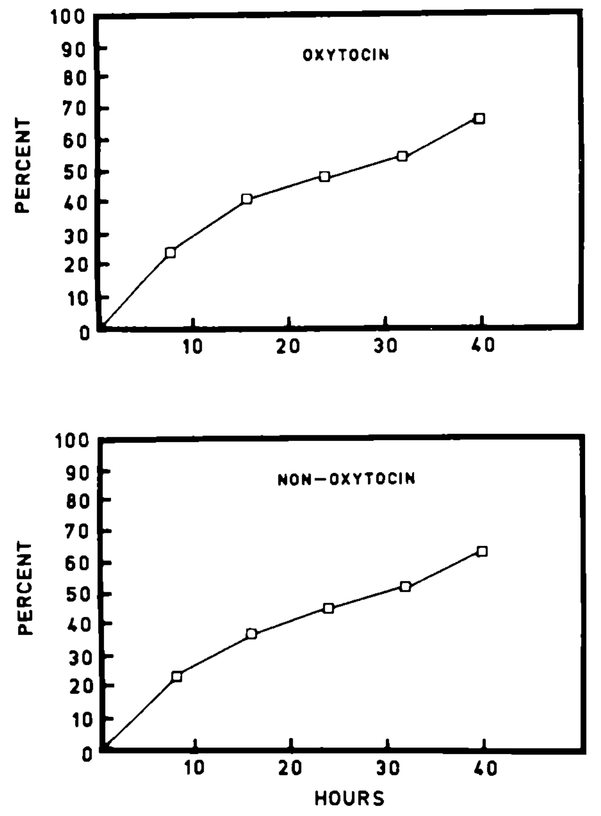

Fig. 3. Percent of administered magnesium excreted in the maternal urine versus time in 18 women who were treated with magnesium sulfate for preeclampsia and oxytocin for induction or augmentation of labor. These results were compared with those in seven women who were treated with magnesium sulfate but did not require oxytocin during labor.

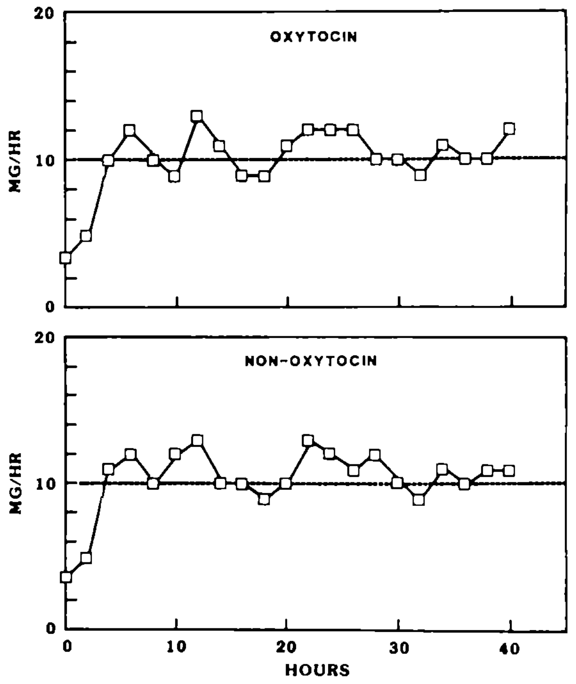

Fig. 4. Urinary calcium excretion rates in 18 women treated with magnesium sulfate for preeclampsia and who received oxytocin for induction or augmentation of labor. These results were compared with those of seven women who were treated with magnesium sulfate but did not require oxytocin during labor.

indirectly proportional to the serum magnesium concentration.[13] The renal magnesium clearance increases as the plasma magnesium concentration rises and hence a decrease in the glomerular filtration rate will decrease magnesium excretion. Similarly, calcium excretion parallels an increase in plasma magnesium levels. This increase in calcium excretion results from a competition between magnesium and calcium for common tubular reabsorptive sites. Thus with the increase in urinary magnesium loss there is a concomitant loss in urinary calcium.[13]

Although oxytocin is frequently used in women receiving magnesium sulfate therapy for preeclampsia, its effects on the pharmacokinetics of magnesium sulfate distribution and excretion are uncertain. In addition to its uterotonic effects, oxytocin is a potent antidiuretic and can cause a decrease in free water clearance and urine output.[14, 15] One could then hypothesize that oxytocin might lead to increased concentrations of magnesium and calcium in maternal serum and cord blood. Magnesium dosage schedules would thus require alteration in those patients concurrently receiving oxytocin. However, despite the theoretical mechanisms by which oxytocin could affect the excretion of magnesium, no adjustment in magnesium sulfate dosage schedules has been prescribed.[11]

The magnesium and calcium concentrations in maternal serum and urine and fetal cord blood reported here are similar to those in previous studies of women receiving parenteral magnesium sulfate therapy without oxytocin.[1-8] The current study demonstrates that concurrent oxytocin administration has no significant effect on the pharmacokinetics of magnesium sulfate therapy. In patients receiving oxytocin compared with those not receiving oxytocin, no significant differences existed in the rates or amounts of magnesium that were excreted throughout the treatment period. In addition, there were no significant differences in the average maternal serum levels during treatment between patients who received oxytocin and those who did not. Fetal cord blood levels were also equivalent.

In conclusion, oxytocin had no significant effect on the pharmacokinetics and distribution of magnesium and calcium in women who had maintenance of a normal urine output. The site of action of oxytocin on the kidney, the distal collecting tubules, is distinct and separate from the sites of magnesium and calcium reabsorption and appears not to alter its excretion or reabsorption. No additional changes in dosage schedules or monitoring of serum levels are required when oxytocin therapy is added in women receiving magnesium sulfate because of preeclampsia.

REFERENCES

1. Pritchard JA. The use of the magnesium ion in the management of eclamptogenic toxemias. Surg Gynecol Obstet 1977;100:131-40.
2. Cruikshank DP, Pitkin RM, Reynolds WA, et al. Effects of magnesium sulfate treatment on perinatal calcium metabolism. I. Maternal and fetal responses. AM J OBSTET GYNECOL 1979;134:243-9.
3. Cruikshank DP, Pitkin RM, Donnelly E, et al. Urinary magnesium, calcium, and phosphate excretion during magnesium sulfate infusion. Obstet Gynecol 1981;58:430-4.
4. Chesley LC, Tepper I. Some effects of magnesium loading upon renal excretion of magnesium and certain other electrolytes. J Clin Invest 1958;37:1362-8.
5. Chesley LC. Parenteral magnesium sulfate and the distribution, plasma levels, and excretion of magnesium. AM J OBSTET GYNECOL 1979;133:1-7.
6. Chesley LC, Tepper I. Plasma levels of magnesium attained in magnesium sulfate therapy for preeclampsia and eclampsia. Surg Clin North Am 1957;37:353-9.
7. Savory J, Monif GRG. Serum calcium levels in cord sera of progeny of mothers treated with magnesium sulfate for toxemia of pregnancy. AM J OBSTET GYNECOL 1971;110:556-9.
8. Sibai BM, Graham JM, McCubbin JH. A comparison of intravenous and intramuscular magnesium sulfate regimens in preeclampsia. AM J OBSTET GYNECOL 1984;150:728-33.
9. Pritchard JA. The use of magnesium sulfate in preeclampsia-eclampsia. J Reprod Med 1979;23:107-14.
10. Brandt JL, Galser W, Jones A. Soft tissue distribution and plasma disappearance of intravenously administered isotopic magnesium with observations on uptake in bone. Metabolism 1958;7:355-61.
11. Pritchard JA, Pritchard SA. Standardized treatment of 154 consecutive cases of eclampsia. AM J OBSTET GYNECOL 1975;123:543-52.
12. Alfrey AC, Miller NL, Butkus D. Evaluation of body magnesium stores. J Lab Clin Med 1974;84:153-62.
13. Carney SL, Wong NLM, Quamme GA, et al. Effects of magnesium deficiency on renal magnesium and calcium transport in the rat. J Clin Invest 1980;65:180-8.
14. Egger TR, Fliegner JR. Water intoxication and Syntocinon intoxication. Aust NZ J Obstet Gynaecol 1979;19:59.
15. Whalley PS, Pritchard JA. Oxytocin and water intoxication. JAMA 1963;186:601-5.

Influence of umbilical vein administration of oxytocin on the third stage of labor: A randomized, double-blind, placebo-controlled study

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A randomized, double-blind, placebo-controlled study evaluated the influence of umbilical vein administration of oxytocin on the third stage of labor. Five minutes after delivery, 37 women received 10 units of oxytocin diluted in physiologic saline solution to a total volume of 20 ml; 41 women received 20 ml of saline solution alone. There was no significant difference between groups in mean (± SD) injection–placental expulsion interval (9 ± 7 versus 10 ± 8 minutes). (AM J OBSTET GYNECOL 1987;157:160-2.)

Key words: Oxytocin, placenta, third stage of labor, umbilical vein

A prolonged third stage of labor may be associated with an increase in hemorrhage and/or a need for manual removal of the placenta. Manual removal, when delayed, often mandates the administration of a general anesthetic. Neri et al.[1] and Golan et al.[2] reported the efficacy of umbilical vein administration of oxytocin in shortening the third stage. Neither of these studies, however, included an appropriate control group. The purpose of the present study was to evaluate the influence of umbilical vein administration of oxytocin on the third stage of labor, with a randomized, double-blind, placebo-controlled study design.

Methods

The protocol was approved by the institutional review board for research involving human subjects. Each patient gave written consent. The study was limited to

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Table I. Obstetric history and conduct of labor

	Oxytocin (N = 37)	*Saline (N = 41)*	*p Value*
Gravidity			
Primigravid	17 (46%)	21 (51%)	NS
Multigravid	20 (54%)	20 (49%)	
Parity			
Nulliparous	20 (54%)	27 (66%)	NS
Parous	17 (46%)	14 (34%)	
Gestational age (wk)*	40.0 ± 1.6	40.0 ± 1.3	NS
Antepartum hemoglobin (gm/dl)*	13.4 ± 1.1	13.1 ± 1.4	NS
Oxytocin augmentation of labor	10 (27%)	13 (32%)	NS
Interval between 4 and 10 cm cervical dilatation (hr)*	4.5 ± 2.8	4.1 ± 2.9	NS
Duration of second stage (min)*	54 ± 47	61 ± 53	NS
Type of delivery			
Spontaneous	29 (78%)	31 (76%)	NS
Low forceps	7 (19%)	9 (22%)	
Midforceps	1 (3%)	1 (2%)	

*Mean ± SD.

women in labor at ≥36 weeks' gestation. Criteria for exclusion included: (1) uterine scar, (2) multiple gestation, (3) chorioamnionitis, (4) abruptio placentae, and (5) marginal placenta previa or low-lying placenta. Also excluded were women who underwent cesarean delivery or in whom the placenta separated within 5 minutes of delivery.

Following delivery, the presence of multiple gestation was excluded by abdominal palpation. If a patient was receiving oxytocin before delivery, the oxytocin was discontinued immediately after delivery. Five minutes after delivery (as determined by the 5-minute Apgar score bell), if there was no evidence of placental separation (that is, change in apparent cord length, vaginal bleeding, and/or uterine shape), 20 ml of coded study solution was injected into the umbilical vein at the vaginal introitus. A hemostat was placed on the umbilical cord just distal to the site of injection so that the solution flowed toward the placenta. After injection, a hemostat was placed just proximal to the site of injection to prevent leakage.

The study solution was freshly prepared by a hospital pharmacist according to a table of random numbers and was administered in a double-blind manner. One group of patients received 10 units of oxytocin (Invenex Laboratories, Chagrin Falls, Ohio) diluted in physiologic saline solution to a total volume of 20 ml. The other group received 20 ml of saline solution alone.

The obstetrician avoided traction on the umbilical cord until there was evidence of placental separation. He/she was allowed to apply suprapubic pressure while observing for evidence of placental separation. However, he/she performed no other intervention to effect placental separation until at least 15 minutes after administration of the study solution. At that time or at any time thereafter, manual removal of the placenta was performed at his/her discretion. After expulsion of the placenta, oxytocin was administered as indicated.

Statistical analysis was by Student's *t* test, Wilcoxon test, χ^2, and Fisher's exact test as indicated. A p value < 0.05 was considered significant.

Results

One hundred eighty-four women consented to participate. Fourteen women were excluded because they underwent cesarean delivery. Eighty-seven women were excluded because the placenta separated spontaneously within 5 minutes of vaginal delivery. Five women were excluded because of a violation of the protocol. Of the remaining 78 women, 37 women received oxytocin, and 41 women received saline solution alone.

The two groups were similar with regard to maternal age, race, socioeconomic status, and weight. Table I includes details regarding obstetric history and conduct of labor.

There was no significant difference between groups in mean (±SD) injection-expulsion interval (9 ± 7 versus 10 ± 8 minutes). One woman in the oxytocin group and two women in the saline group required manual removal of the placenta. There was no significant difference between groups in mean (±SD) second-day-postpartum hemoglobin level (11.6 ± 1.2 versus 11.5 ± 1.4 gm/dl). One woman in the oxytocin group and no women in the saline group received a blood transfusion.

Comment

Golan et al.[2] administered 10 units of oxytocin (diluted in 20 ml of saline solution) into the umbilical vein in 10 women, each of whom had a retained placenta at 30 minutes after delivery. The mean injection-expulsion interval was 3.7 minutes. Two of the 10 patients required manual exploration of the uterine cavity after expulsion of the placenta. Neri et al.[1] had earlier given 5 units of oxytocin (diluted in 10 ml of saline solution) into the umbilical vein in 330 parturient women immediately after delivery. The mean injection-expulsion interval was 3.3 minutes; eight patients subsequently underwent uterine exploration. In the present study, umbilical vein administration of oxytocin was delayed until 5 minutes after delivery, as a significant percentage of patients can be expected to undergo rapid, spontaneous separation of the placenta.

Both Neri et al.[1] and Golan et al.[2] contended that this procedure facilitates the delivery of a high concentration of oxytocin to the placental bed and uterine wall, resulting in uterine contraction and placental separation. However, we conclude that, under the conditions of the present study, routine administration of 10 units of oxytocin (diluted in physiologic saline solution), compared with administration of 20 ml of saline solution alone, does not significantly shorten the third stage of labor. We acknowledge that the present study does not address the possibility that injection of saline solution alone may be superior to expectant management.

REFERENCES

1. Neri A, Goldman J, Ganz B. Intra-umbilical vein injection of Pitocin: a new method in the management of the third stage of labor. Harefuah 1966;70:351-3.
2. Golan A, Lidor AL, Wexler S, David MP. A new method for the management of the retained placenta. AM J OBSTET GYNECOL 1983;146:708-9.

Echocardiographic and anatomic studies of the maternotrophoblastic border during the first trimester of pregnancy

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The initiation of uteroplacental circulation was investigated in early pregnancy with the following material: contact ultrasonography, 250 cases; direct-vision chorionic biopsy, 10 cases; products from voluntary interruption of pregnancy, 60 cases; and hysterectomy with the pregnancy in situ, three cases. Using all of these techniques, we were unable to demonstrate a true intervillous blood flow during the first 12 weeks. We suggest that during this period the intervillous space is bathed by an acellular fluid that could be plasma filtered by the trophoblastic shell and its endovascular cone. If this is the case, the physiology of early pregnancy must be reconsidered. (AM J OBSTET GYNECOL 1987;157:162-8.)

Key words: Early pregnancy, trophoblastic shell, spiral arteries, uteroplacental blood flow, contact ultrasound–chorionic villus sampling

Ultrasonic studies dealing with early pregnancy have until recently focused on objective criteria of embryonic vitality, that is, morphologic findings and biometry of the gestational sac and embryo and evaluation of embryonic cardiac activity and embryonic movement.[1] All of these criteria have led to a very accurate dating of the pregnancy (±3 days).[2, 3] This accuracy has recently been significantly increased by the use of vaginal probes.[4, 5] However, the interest of the ultrasonographer has not centered on the demarcation and/or interrelationship between endomyometrium and trophoblast, probably because of a lack of sufficient resolution. Contact ultrasonography is, in this respect, a powerful tool.[2]

According to the classic theories,[6, 7] the intervillous space is occupied by maternal blood from the twenty-second day onward. This statement has always been taken as sufficient proof of a real circulation, since the rapidly growing embryo needs an important supply of nutrients. For the past 3 years our department has been intensely involved in chorionic villus sampling by means of forceps biopsy via the cervical route. Surprisingly, we observed that almost no accurate samples were ever blood tinged or followed by any blood leakage, although the intervillous space was obviously entered. Thus we felt obliged to reconsider the classic

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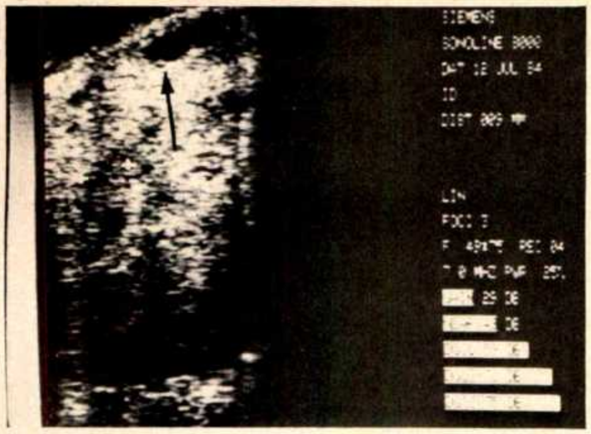

Fig. 1. Contact echography diameter of the gestational bag (9 mm) corresponding to a 5½-week pregnancy. A large uterine vessel is seen at some distance *(arrow)*.

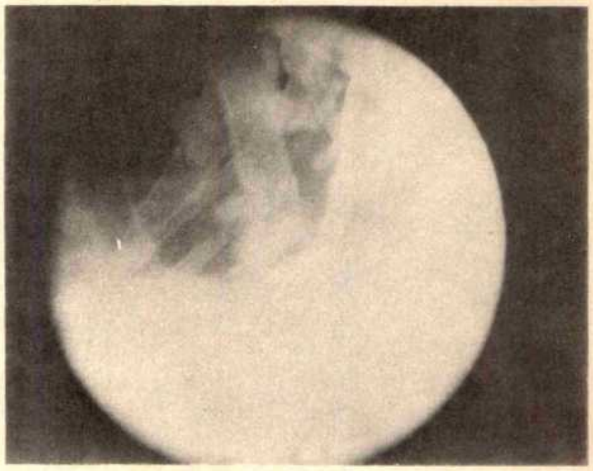

Fig. 2. In vivo hysteroscopy in a 9-week pregnancy Villi appear as "whitish worms." No blood discoloration is present.

theories and wondered if the intervillous space is effectively occupied by maternal blood during most of the first trimester.

In this article we present the results of studies with contact ultrasonography, as well as direct-vision hysteroscopy. Moreover, we will introduce morphologic evidence that the early placenta is bathed by fluid that is not completely maternal blood.

Material and methods

Ultrasound studies. Two hundred fifty normal pregnancies have been studied between the fifth and twelfth week of gestation by means of a Sonoline 8000 (Siemens AG, Erlangen, Germany) apparatus coupled with a miniaturized vaginal linear array that is made up of 57 crystals for a total length of 4.5 cm and a width of 1.2 cm. The ultrasound frequency was 7 mHz. The frame rate was 24/sec. All investigations were recorded on videotape. All patients gave their informed consent for the procedure. The vaginal insertion was performed during a routine examination. The probe was placed in the vaginal fornices. Bladder filling was unnecessary. In most patients a second vaginal ultrasound examination was performed after an interval of 2 to 4 weeks.

Morphologic studies

Hysteroscopy. Ten patients who were scheduled for termination of pregnancy between 8 and 10 weeks were submitted to a hysteroscopic procedure after informed consent. The apparatus (Hysteroscope Storz AG of 4 mm outer diameter) was introduced through the cervical canal under general anesthesia. Irrigation of the uterine cavity was initiated under low pressure until the optics were clearly positioned in the intervillous space. The saline solution drip was then stopped, villous samples were obtained by direct-vision biopsy, and photographs were systematically recorded. After completion of the procedure, evacuation of the uterine contents was performed by either suction or dilatation and curettage. Products thus obtained were immediately fixed in buffered formalin and processed for routine histologic examination.

First-trimester placentas. Sixty first-trimester placentas were obtained between the seventh and twelfth weeks after uneventful pregnancies that had been legally terminated. The specimens were fixed in buffered formalin embedded in Paraplast (Monoject, Kildare, Ireland) and were routinely sectioned at 5 μm and stained with hematoxylin-eosin.

Three pregnant patients underwent a hysterectomy during this observation period. The first case was a 29-year-old woman who had carcinoma in situ of the cervix. The pregnancy was 9 weeks. After ultrasonographic orientation, the uterus was opened opposite the placental insertion site and immersed in a large volume of formalin. It was subsequently cut into blocks numbered consecutively and serially sectioned.

The second case was a 38-year-old woman who had a hysterectomy at 10 weeks of gestation for several intramural myomas. The specimen was processed in the same fashion as the first one. The third case was a 36-year-old woman who was 9 weeks pregnant. After hysterectomy, both uterine arteries were cannulated, flushed with heparinized saline solution, and then perfused at a mean pressure of 100 mm Hg with a barium sulfate suspension (Micropaque, Nicholas S.A., Brussels, Belgium) diluted 1:3 with buffered 4% formalin. When perfusion was complete, that is, when the barium suspension appeared at the uterine veins, all vessels were tied, and the uterus was immersed in several liters of buffered formalin. After 10 days the specimen underwent x-ray examination, was sliced (1 cm thick), and again was submitted to x-ray examina-

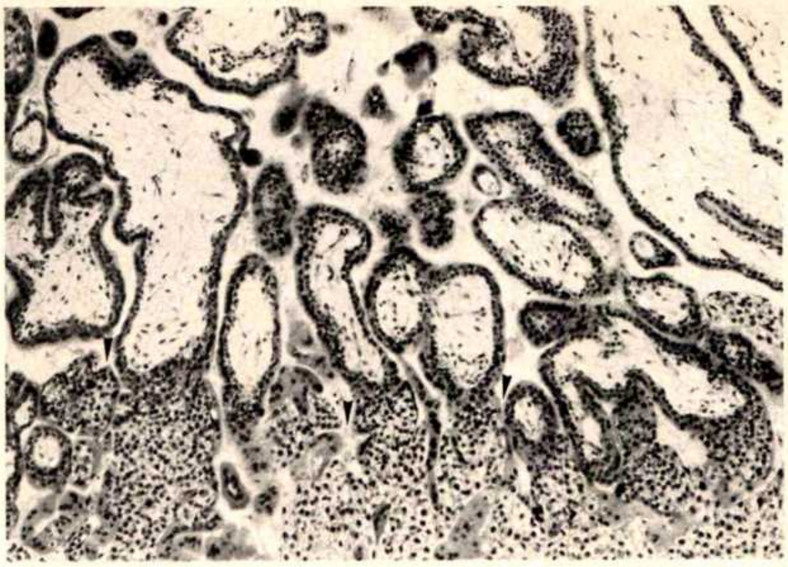

Fig. 3. Eight-week pregnancy voluntarily interrupted. Trophoblastic shell *(arrows)* is fused with cytotrophoblastic cell columns. (Hematoxylin-eosin stain; original magnification ×100).

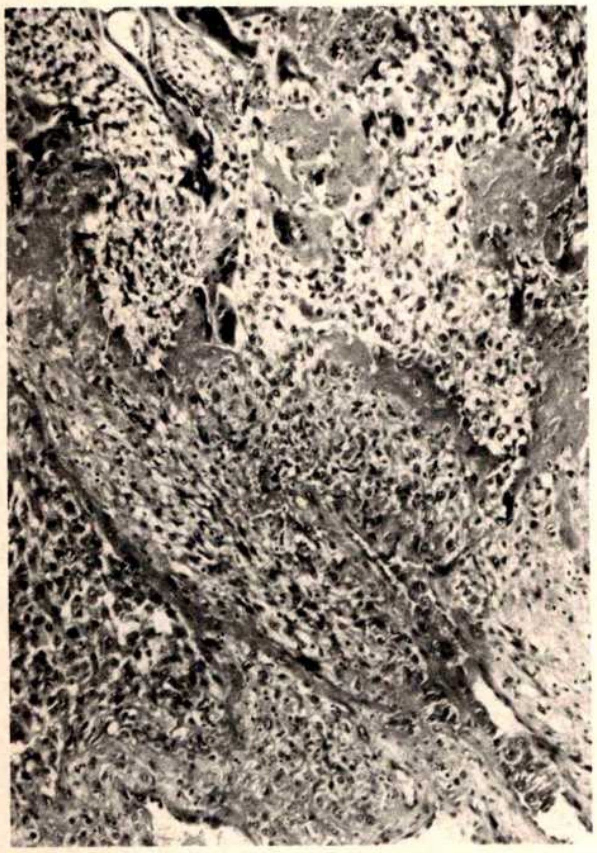

Fig. 4. Eight-week pregnancy (voluntary interruption). Maternal side of the trophoblastic shell *(arrows)*. A trophoblastic cone extends just beneath in a dilated spiral artery. (Hematoxylin-eosin stain; original magnification ×250.)

tion. It was subsequently processed for routine histologic examination as the first two specimens were processed.

Results

Ultrasonography with a vaginal probe. The physical properties of the vaginal probe allowed a clear definition of anatomic structures of 1 mm width in the explored field (real-time ultrasound examination). It was possible to detect the presence of a gestational sac at 4½ weeks of gestation (calculated from the first day of the last menstrual period). It appeared as a small echo-free area (1 cm average diameter) surrounded by an echo-dense ring. This sac was located separately from the endometrial echoes and could thus be easily separated from an enlarged uterine cavity. Embryonic cardiac activity could be detected when the cardiac primary tube began to pulse (twenty-second to twenty-third day of life, 5½ weeks' gestational age, and crown-rump length of 3 mm). Uterine vessels were evident approximately 1 cm apart from the trophoblastic ring (Fig. 1). Small-moving echoes were usually identified; they were synchronous to the maternal pulse. As pregnancy advanced, the vascular network became more conspicuous and grew closer to the trophoblastic shell. The latter also changed; it thickened in the region of the future placenta around the funicular merging with the uterine wall. However, uterine vessels did not reach the placenta. It was impossible to define any particulate movement in the trophoblastic area during the period of observation.

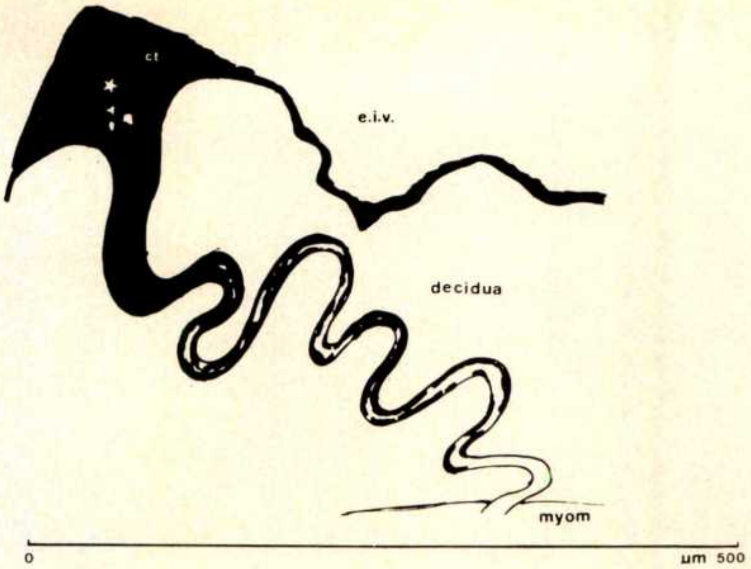

Fig. 5. Hysterectomy specimen with 9-week pregnancy in situ. Reconstruction from serial sections of the course of spiral artery between the deciduomyometrial junction and its opening in the trophoblastic shell. Average length irrespective of numerous coils is 500 to 700 µm. *e.i.v.* = Intervillous space.

Morphologic findings. When the hysteroscope was introduced into the uterine cavity, it could easily be positioned in the intervillous space by ultrasound guidance, provided that a limited amount of saline solution was perfused at low pressure through the outer tube. In the 10 patients observed, hysteroscopic observation could demonstrate villi as whitish "worm-like" structures (Fig. 2). It was possible to see embryonic vessels within the villus core that appeared as pinkish threads. We did not observe blood discoloration in the intervillous space, and the perfusion could be stopped at the entrance into the intervillous space with an intact view of the contents. No bleeding occurred after withdrawal of the hysteroscope. The villus samples obtained by direct-vision biopsy were immediately coverslipped and examined in the fresh state. The morphologic findings were characteristic of the first trimester. Nucleated red blood cells were occasionally encountered.

Products of conception after termination of pregnancy. Our objective was not the description of first-trimester villi but the study of the maternal embryonic relationship. In all subjects, good variability of the placenta and embryo was suggested by a normal appearance of the histologic structures. Since disruption of the intervillous space with curettage is inevitable, no attention was paid to the presence of maternal blood. Immediate interest was focused on the implantation site and the uteroplacental arteries. The trophoblastic shell appeared to be continuous until 10 weeks of gestation. It was covered on both sides with spikes: On the fetal side they consisted of trophoblastic cell columns (future anchoring villi), whereas on the maternal side the intravascular trophoblastic plugs were intimately sealed with the shell (Figs. 3 and 4). These findings were characteristic only of the true placental site. The chorion laeve essentially consisted of regressing villi with wandering trophoblasts covered with a very limited amount of decidua. Necrotic areas were more extensive in the chorion laeve.

It was interesting to note that most normal products of conception had a cleavage line that followed exactly the outer side of the trophoblastic shell at a pole opposite to the implantation site. Under the trophoblastic shell, no continuous fibrinoid layer could be demonstrated. Fibrin deposition was only conspicuous in those areas in which arterial thrombosis and decidual infarcts were present. In all subjects, trophoblastic invasion of the decidua was bimodal; an important number of isolated cells penetrated interstitially, whereas cellular plugs, which were sometimes rather loose, extended into the dilated spiral arteries.

Hysterectomy specimens. Serial sections on selected blocks allowed us to follow the course of the spiral arteries. Most ran a course parallel to the maternal placental interface for 200 to 300 µm and then became coiled while they progressively reached this interface. The opening of the uteroplacental artery was occluded by a trophoblastic plug. This plug extended toward the decidual myometrial junction, but in several sections, the vascular lumen was patent. Moreover, at the level

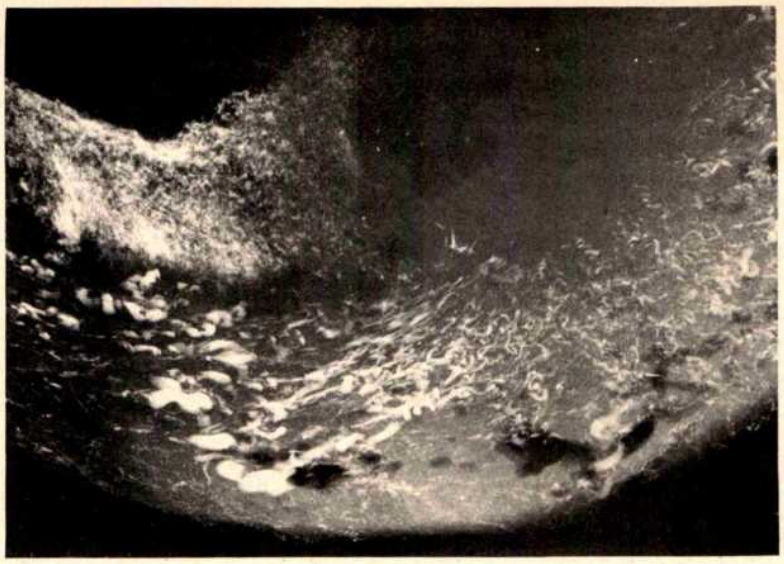

Fig. 6. Hysterectomy specimen with pregnancy in situ and both uterine arteries injected with radiopaque medium. Outer limits of the insertion site. The decidua encircling the early placenta is copiously vascularized while the intervillous space is devoid of any contrast medium. (× 3 enlargement from x-ray film slides of transversal sections 1 cm thick.)

of the terminal coils, several slits could be identified that laterally connected the spiral artery (most often occluded by trophoblasts) with the trophoblastic shell.

Red blood cells were present between trophoblastic cells. They were also found in the slits and in some lacunar spaces of the trophoblastic shell but not in the intervillous space. The average length of the spiral arteries was 500 to 750 μm in the decidua of the insertion site notwithstanding the numerous coils (Fig. 5).

Studies of x-ray films from one uterus injected with barium sulfate were of utmost interest. The intervillous space was devoid of radiopaque medium. In the 1 cm thick slices, small vessels were numerous at the insertion site and particularly at its lateral borders (Fig. 6). On histologic slides, the spiral arteries were filled with barium sulfate. They were distended with a loosening of the trophoblastic plugs that intermingled with the endothelial layer. Particulate barium was identified in some lacunae of the trophoblastic shell that communicated with the vascular lateral slits.

Comment

Maternal red blood cells have been detected very early in the primitive intervillous space, probably around the tenth to eleventh day after the period of conception. These cells are trapped in the trophoblastic lacunae and originate from circumferential distended sinusoids.[7] Classically, more and more maternal vessels are progressively tapped, and according to Boyd and Hamilton[6] and Kaufmann,[7] a true blood flow is established around the twenty-ninth day. Our findings do not concur with this theory.

In vivo contact ultrasonography was unable to delineate moving echoes in the intervillous space before week 13 in patients with normally-evolving pregnancy. It must be noted that this technique has not, to the best of our knowledge, been previously applied to a systematic study of early fetoplacental development. Real-time ultrasound examination can pick up the motion of blood cells by the phenomenon of ultrasound energy diffusion on moving cells. Moreover, chorionic villus sampling under direct-vision biopsy brought immediate proof that there is no true blood with hemoglobin-loaded erythrocytes surrounding the villi. These observations have been seen repeatedly. It should be noted that in all patients saline solution infusion was stopped when the optics entered the intervillous space, thus suppressing any excess pressure. Our morphologic studies may offer an explanation. During the first 12 weeks, the trophoblastic shell is completely tight and is bristling on both sides, that is, maternal and fetal, with numerous cellular cones (Fig. 7). On the fetal side these consist of trophoblastic cell columns that proliferate actively; on the decidual side they are made up of intravascular trophoblastic plugs that can extend up to the decidual myometrial junction at 9 to 10 weeks.[8] According to our hypothesis, the growing embryo and placenta are completely separated from the maternal milieu during perhaps the whole first trimester. There is no or very limited contact with maternal cells during this period except at the deciduotrophoblastic interface

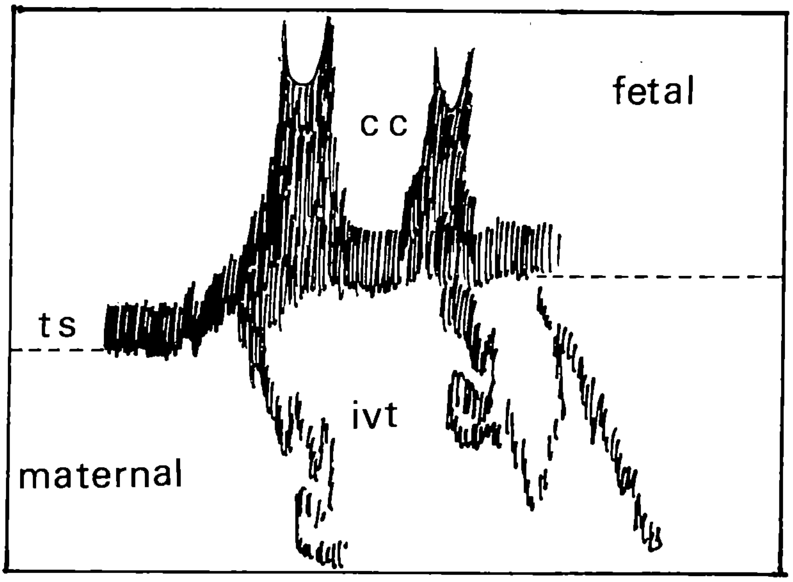

Fig. 7. Schematic representation of the peripheral trophoblastic layer during the first 12 weeks. It must be emphasized that trophoblastic cell columns *(cc)*, trophoblastic shell *(ts)*, and endovascular *(ivt)* trophoblastic plugs *(cones)* form a continuum, theoretically without leakage, which protects the organizing embryo and growing placenta from most external influences.

and in the spiral arteries. Ramsey and Donner[9] questioned the exact period during pregnancy when a true blood flow was established. However, their experiments on primates indicated that it would occur late.

Pijnenborg et al.[8] have described the intravascular trophoblastic invasion. They also mentioned the existence of lateral slits communicating with the trophoblastic shell. However, they did not consider that a markedly reduced or even arrested blood flow was a necessary consequence of this anatomic structure.

We find it remarkable that in our hysterectomy specimen injected with a radiopaque dye, the mean pressure at the uterine artery level was 100 mm Hg, and despite a sustained flow lasting about 30 minutes, there was no particulate barium sulfate in the intervillous space. The uterine veins, however, were filled with contrast media, as were the spiral arteries, up to the trophoblastic cones. One might speculate about the possible existence of arteriovenous shunts in or near the placental insertion site, possibly at the deciduomyometrial interface.

The most important problem in early (i.e., first trimester) placental development resides in the establishment of equilibrium between the intrauterine expansion of the growing placenta and the effectiveness of the trophoblastic shell as a temporary barrier. This dynamic equilibrium cannot exist if maternal blood is allowed to spurt freely into the intervillous space. At this early stage of pregnancy, anchoring villi have not yet reached the effectiveness of the late stages since they are only made of cellular, that is, trophoblastic expansions of the outer shell and thus are easily torn off if pressure rises even minimally. One might speculate that this may play a role in spontaneous abortions.

Nevertheless, we do not want to state definitively that during the first 12 weeks the intervillous space is absolutely bloodless. Some leakage may exist; however, in general it seems that all wandering maternal cells remain trapped within the trophoblastic shell, which acts as a cell filter.

Thus it follows that most of the intervillous space is occupied by a fluid that in essence must be plasma with a possible but limited admixture of uterine gland secretions. Our evidence would seem to indicate this; however, absolute proof will be present only when the exact composition of the fluid is known.

If our hypothesis proves valid, it raises many questions, the most important of which are: (1) Under the conditions described, how can the fuel supply to the growing embryo be sufficient? (2) At the crucial moment when organogenesis takes place, is it conceivable that the oxygen supply to the conceptus would not be fully hemoglobin mediated but on the contrary, would be dependent on the partial pressure of oxygen as defined by its solution in plasma? If this is true, then embryonic organogenesis must proceed under a reduced oxygen tension.

It is already well known that the first cleavage steps of the fertilized ovum are best accomplished under reduced oxygen pressure.[10] We are convinced that our observations closely mirror reality and that new ways of thinking are needed to explain the physiology of early gestation. In particular, we suggest that there are two periods. The first lasts about 12 weeks and is when implantation, organogenesis, and building of the definitive placenta take place. All of these events that encompass vertebrate phylogeny in a very short time could occur without maternal blood flow, that is, in low oxygen-tension conditions, resembling those conditions that occur in fish embryos that have external gills (i.e., the early villus placenta). The second period would encompass the last two thirds of pregnancy, which are fully characteristic of mammalian evolution, and during which fetal growth occurs with increased requirements that eventually need an active maternal blood flow in the intervillous space. Much of this, of course, is highly speculative. Several studies are currently being conducted to develop additional evidence.

We thank Dr. E. Ramsey for the fruitful discussions on our hypothesis.

REFERENCES

1. Gaspard U, Schaaps JP, Piront F, et al. Valeur pronostique des taux sériques maternels de la choriogonadotrophine et de ses unités libres alpha et beta dans la menace d'avortement du premier trimestre. Corrélation avec l'examen ultrasonographique. J Gynecol Obstet Biol Reprod (Paris) 1980;9:62.
2. Robinson HP. Sonar measurement of fetal crown rump length as means of assessing maturity in first trimester of pregnancy. Br Med J 1973;4:28.
3. Robinson HP, Fleming JEE. A critical evaluation of sonar crown-rump length measurements. Br J Obstet Gynaecol 1975;82:702.
4. Schaaps JP. Endo-échographie en gynécologie-Obstétrique. J Echog Med Ultrason 1985;6:275.
5. Schaaps JP, Lambotte R. Ultrasonic observation of pregnancy during the first trimester, using a vaginal approach. In: Fraccaro M, Simoni G, Brambati B. et al., eds. First trimester fetal diagnosis. Berlin: Springer-Verlag, 1985:78.
6. Boyd JD, Hamilton WJ. The human placentae. Cambridge, England: W. Heffer Sons Ltd, 1970:253.
7. Kaufmann P. Entwieklung der Plazenta. In: Becker V, Schiebler TH, Kubli F, eds. Die Plazenta des Menschen. Stuttgart: Georg Thieme Verlag, 1981:13.
8. Pijnenborg R, Dixon G, Robertson WB, Brosens I. Trophoblastic invasion of human decidua from 8 to 18 weeks of pregnancy. Placenta 1980;1:3.
9. Ramsey EM, Donner MW. Placental vasculature and circulation. Stuttgart: Georg Thieme Verlag, 1980:90.
10. Quinn P, Harlow GM. The effect of oxygen on the development of preimplantation mouse embryos in vitro. J Exp Zool 1978;206:73.

Human embryo viability related to freezing and thawing procedures

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Freezing and thawing procedures did not affect the chances of successful transfer of human embryos. The postimplantation viability of frozen-thawed embryos was equivalent to that of fresh, immediately transferred embryos. Selection of the embryos that were more suitable for freezing did influence the pregnancy rate after transfer of the remaining fresh embryos. The therapeutic efficacy of in vitro fertilization and embryo transfer programs will be greatly increased if only one embryo is transferred in the in vitro fertilization cycle, the others being separately frozen for further embryo transfer in subsequent cycles. (AM J OBSTET GYNECOL 1987;157:168-71.)

Key words: In vitro fertilization–embryo transfer, embryo freezing

We have previously described a method of human embryo freezing with 1,2-propanediol used as a cryoprotectant.[1] The first results after transfer of frozen and thawed embryos have demonstrated that it is possible to obtain a high pregnancy rate by including embryo freezing in an in vitro fertilization and embryo transfer program.[2]

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The choice of which embryo(s) to freeze from those obtained in a single in vitro fertilization cycle depends on two criteria: the cleavage stage of each embryo and its appearance at the time when the other embryos are transferred. It is necessary to demonstrate that embryo freezing according to this practice does not diminish the success rate that is seen with fresh embryo transfer.

Another problem is the evaluation of the best chance to obtain a pregnancy according to the policy of embryo freezing, that is, how many embryos have to be transferred immediately and how many have to be frozen for further transfer(s) after thawing. However, to adequately perform this calculation, we must consider all frozen fertilized eggs and not just those that survive the freezing and thawing procedures.

It was the aim of the present study to answer both of these questions from the preliminary results obtained in 124 in vitro fertilization cycles in which embryo(s) were frozen and thawed.

Material and methods

Patients and treatments. All women were treated in our in vitro fertilization–embryo transfer unit according to several protocols for ovarian stimulation during the cycle of oocyte recovery. Follicle growth was induced by clomiphene citrate (Clomid, Merrell Toraude, Paris, France) and human menopausal gonadotropin (hMG; Inductor, Searle, Paris, France) in monitored cycles as previously described[3] or in programmed cycles.[4] Programmed cycles represented 20% of cycles in which no embryos were frozen and 50% of cycles in which certain embryos were frozen. Oocyte recovery took place 35 hours after human chorionic gonadotropin (hCG) administration.

Oocyte and embryo culture. The methods used for sperm preparation, for in vitro insemination, and for embryo culture and transfer have already been described.[5,6] Briefly the entire culture was performed inside a modified newborn incubator (Wild-Leitz, Rueil, 95506, France) with the use of Menezo B_2 culture medium (Api System, La Balme les Grottes, 38390, France) free of serum.

Embryo freezing and thawing procedures. After dehydration with 1,2-propanediol (1.5 mol/L) and sucrose (0.1 mol/L) the embryo was introduced into a plastic straw to be frozen in a programmed freezer (Minicool, CFPO, Sassenage, 38360, France). Only 1- or 2-day embryos were frozen. We intended to freeze mainly embryos at the pronucleated stage or those containing two or four regular blastomeres without any cytoplasmic exudate.[1] The duration of cryopreservation was between 1 and 15 months. Embryo transfer was performed in the patient from whom the oocyte(s) were obtained and took place either in a natural cycle (n = 62) or in a cycle treated with hMG (n = 11) or hCG (n = 3) or hMG plus hCG (n = 24). The embryos were introduced into the uterus at a time after ovulation that was equivalent to their developmental age after in vitro insemination.[2]

Table I. Pregnancy rate in in vitro fertilization cycles excluding embryo freezing

	No. of transferred embyos		
	1	*2*	*3*
Transfer cycles (n)	210	166	129
Pregnancies			
n	25	34	27
%	11.9	20.5	20.9

Table II. Pregnancy rate after fresh embryo transfer in in vitro fertilization cycles in which other embryos were frozen

	No. of transferred embryos		
	1	*2*	*3*
Transfer cycles (n)	27	25	95
Pregnancies			
n	10	4	26
%	37.0	16.0	27.4

Policy for embryo freezing. The policy for embryo freezing was not clearly defined until the end of this preliminary study. In some patients no embryos were transferred in the in vitro fertilization cycle whereas one to three embryos were immediately transferred in others. The number of frozen embryos was one to 11 per in vitro fertilization cycle.

Selection of control subjects. Two groups of patients having fresh embryo transfer were used as controls for the evaluation of the efficiency of the freezing method. There were 505 in vitro fertilization cycles in which all embryos were immediately transferred into the uterus and 147 in vitro fertilization cycles in which certain embryos were immediately transferred whereas others were frozen. All of these in vitro fertilization cycles took place during the same period of study.

Results

In the 505 in vitro fertilization cycles in which no embryos were frozen, pregnancy rates were 11.9%, 20.5%, and 20.9% after the transfer of one, two and three fresh embryos, respectively (Table I). In the 147 in vitro fertilization cycles where one or several embryos were frozen, there were 37.0%, 16.0%, and 27.4% pregnancies after the transfer of one, two, and three fresh embryos, respectively (Table II). After single fresh embryo transfers the pregnancy rate was therefore higher when other embryos were frozen (37.0% versus 11.9%, $p < 0.001$). There was no influence of the type of ovar-

Table III. Pregnancy rate after frozen-thawed embryo transfer

	No. of transferred embryos		
	1	*2*	*Total*
Transfer cycles* (n)	76	24	100
Pregnancies			
n	18	8	26
%	23.7	33.3	26.0

*Only embryos that kept 50% of their initial number of cells.

Table IV. Outcome of pregnancies induced by transfer of fresh or frozen embryos

	Transferred embyros	
	Fresh	*Frozen-thawed*
Beginning pregnancies (n)	126	26
Biochemical abortions (n)	17	4
Clinical abortions (including ectopic pregnancies) (n)	19	5
Ongoing pregnancies		
n	90	17*
%	71.4	65.4

*From which 17 normal babies were delivered.

ian stimulation on pregnancy rate after fresh embryo transfer (18.8% and 19.4% for programmed and monitored in vitro fertilization cycles, respectively).

There were 130 in vitro fertilization cycles in which frozen embryos were thawed. The total number of frozen-thawed embryos was 193, from which 124 (64.2%) kept at least 50% of their initial number of cells and were transferred. There were 76 single and 24 double embryo transfers resulting in 18 (23.7%) and eight (33.3%) pregnancies, respectively (Table III).

The pregnancy rate per in vitro fertilization cycle was 19.5%, 21.6%, and 33.3% according to freezing and thawing of one, two, and three embryos respectively regardless of embryo survival after thawing. These figures did not differ from those obtained when the embryos were immediately transferred (Tables I and II). When a pregnancy began, its progress was not influenced by the origin of the transferred embryos (fresh or frozen-thawed, Table IV). The percentages of ongoing pregnancies were 71.4% and 65.4% after the implantation of fresh and frozen-thawed embryos, respectively.

Comment

From these preliminary data two important facts emerge. First, frozen embryos have the same chance to initiate a pregnancy as fresh embryos despite the fact that one third of them do not survive the freezing-thawing procedures. We can therefore postulate that certain lost embryos would not have resulted in successful pregnancy even if not frozen. It is possible that the freezing-thawing procedures exert a selective effect and that only the viable embryos can survive. It appears as if the majority of embryos that are lost are those not suitable for freezing. Embryo survival after thawing was twice as high for embryos judged to have a favorable appearance, regular blastomeres, and no exudate as for those without these features.[6] We have also observed a very low survival rate for embryos frozen at the three-cell stage or after the six-cell stage with our freezing-thawing conditions.[6] It can also be stated that the post-implantation viability of frozen embryos is equivalent to that of fresh embryos as the ongoing pregnancy rate was comparable in both cases.

The second fact to be demonstrated by this study is that the selection of certain embryos for freezing does not have a deleterious effect on the outcome after transfer of the remaining fresh embryos. This justifies the selection of the most suitable embryos for freezing on the basis of their morphologic appearance and their developmental stage.

Three factors are generally acknowledged to affect the success of embryo transfer. The quality of the embryo cannot be improved by cryopreservation but, as discussed above, it is possible to select the most appropriate for this procedure. There were more programmed cycles in the group of patients with embryo freezing than in the control group. Although the mode of ovarian stimulation in the recovery cycle may theoretically affect embryo quality no difference was found in the pregnancy rate after fresh embryo transfer in programmed or monitored cycles. The second factor is the quality of the luteal phase and this can be improved by using the natural or lightly stimulated cycle for frozen embryo transfer. This may explain in part the relative success of frozen compared with fresh single embryo transfer (23.7% and 11.9%, respectively). The third factor that may influence the success of embryo transfer is the technical aspects of the transfer procedure. Cryopreservation enables us to multiply the number of transfers for a given number of available embryos. Freezing the embryos in individual straws not only increases the theoretical possibility of pregnancy[7] but also avoids the risk of multiple pregnancy.

It is obvious that all these improvements depend on a high success rate for embryo cryopreservation procedures. Our embryo freezing method appears several times more efficient than those reported by others[8, 9] if related to the pregnancy rate per fertilized egg excluded from immediate transfer in the in vitro fertilization cycle. This may be partly explained by the poor developmental ability of 2- or 3-day embryos when dimethylsulfoxide is used as cryoprotectant[8] and by the deleterious effect of in vitro conditions if the eggs need to be cultured for 4 or 5 days before being suitable for freezing when glycerol is used as the cryoprotectant.[9]

From the results reported here it is urgent to reconsider the definition of a supernumerary embryo obtained in an in vitro fertilization cycle. Our opinion is that the therapeutic efficacy of in vitro fertilization–embryo transfer will be greatly increased if only one embryo is transferred in the in vitro fertilization cycle, the others being separately frozen for further embryo transfer in subsequent cycles.

REFERENCES

1. Lassalle B, Testart J, Renard JP. Human embryo features which influence the success of cryopreservation with the use of 1,2 propanediol. Fertil Steril 1985;44:645-51.
2. Testart J, Lassalle B, Belaisch-Allart J, et al. High pregnancy rate after early human embryo freezing. Fertil Steril 1986;46:268-72.
3. Belaisch-Allart J, Frydman R, Testart J, et al. In vitro fertilization and embryo transfer program in Clamart, France. J In Vitro Fert Embryo Transfer 1984;1:51-5.
4. Frydman R, Rainhorn JD, Forman R, et al. Programmed oocyte retrieval during routine laparoscopy and embryo cryopreservation for later transfer. AM J OBSTET GYNECOL 1986;155:112-7.
5. Testart J, Lassalle B, Frydman R. Apparatus for the in vitro fertilization and culture of human oocytes. Fertil Steril 1982;38:372-5.
6. Testart J, Lassalle B, Forman R, et al. Factors influencing the success rate of human embryo freezing in an in vitro fertilization and embryo transfer program. Fertil Steril (in press).
7. Testart J, Lassalle B, Belaisch-Allart J, Hazout A, Frydman R. Human embryo freezing in an IVF and ET programme. In: Feichtinger W, and Kemeter P, eds. Future aspects in human in vitro fertilization. Berlin: Springer-Verlag, 1987:91-6.
8. Freeman L, Trounson A, Kirby C. Cryopreservation of human embryos: progress on the clinical use of the technique in human in vitro fertilization. J In Vitro Fert Embryo Transfer 1986;3:53-61.
9. Cohen J, Simon RS, Fehilly CB, Edwards RG. Factors affecting survival and implantation of cryopreserved human embryos. J In Vitro Fert Embryo Transfer 1986;3:46-52.

The effects of aminophylline and nifedipine on contractility of isolated pregnant human myometrium

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To characterize the effects of aminophylline and nifedipine on pregnant human myometrium, in vitro contractility studies were performed on myometrial strips obtained at cesarean section. The strips were stimulated with oxytocin (800 mU/L) to simulate labor and then were exposed to increasing concentrations of aminophylline (40, 100, and 400 μmol/L) or nifedipine (5, 10, and 20 μg/L). Both drugs produced a dose-related decrease in contraction strength, as measured by the time-integrated force of contraction. Aminophylline lengthened the period of contraction in a manner that was not dose dependent. Low-dose nifedipine (5 μg/L) increased the period of contraction, but higher doses had no effect on frequency. Both drugs produced a net reduction in the effectiveness of labor, as measured by the average force (time-integrated force divided by period). These results indicate that both aminophylline and nifedipine may be clinically useful tocolytic agents. (AM J OBSTET GYNECOL 1987;157:171-7.)

Key words: Aminophylline, nifedipine, myometrium, pregnant human myometrium

Prematurity causes significant neonatal morbidity and mortality at a considerable expense to the health care system. Pharmacologic uterine relaxation is intended to result in pregnancy retention, allowing greater fetal maturity to be attained. Currently, β-adrenergic receptor agonists are the only agents approved for use in preterm labor. However, the usefulness of these drugs may be limited by their side effects, which include tachycardia, hypotension, hyperglycemia, electrolyte imbalances, and pulmonary edema. Drugs other than the β-adrenergic receptor agonists may produce adequate tocolysis with fewer toxic side effects. This study characterizes the actions of two such potential tocolytic agents by examining their abilities to

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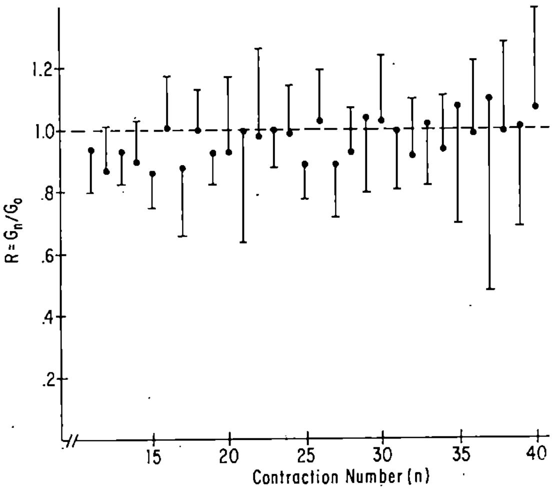

Fig. 1. Effect of oxytocin stimulation on average force as a function of contraction number. Each point represents the average ± SD of six experiments.

relax human myometrium in vitro. We report the effects of aminophylline, a phosphodiesterase inhibitor, and nifedipine, a calcium channel antagonist, on the rate and force of contractions of pregnant human myometrium under oxytocin stimulation.

Material and methods

Informed consent was obtained from pregnant patients, ranging from 27 weeks' gestation to term, undergoing cesarean section in the Department of Obstetrics and Gynecology at Duke University Medical Center. Small segments of uterus were cut from the upper margin of the uterine incision during low transverse cesarean delivery. The tissue was rapidly immersed in Hepes-buffered physiologic salt solution (see below) containing excess lidocaine (0.2 to 0.5 mg/ml) for preservation. The tissue samples were used immediately or stored at 4° C until the next day.

Strips of myometrium, measuring approximately 2 by 2 by 15 mm, were dissected, with the long axis of the collagen fibers oriented along the long axis of the strips. Each strip was rinsed free of lidocaine and anchored in a 20 ml jacketed glass organ bath (Harvard Apparatus, South Natick, Massachusetts) with 4-0 silk suture. The strips were placed under 500 mg initial tension and began contracting spontaneously within 30 to 150 minutes. Isometric tension was measured with a Grass Ft 03 force displacement transducer and recorded on a Grass polygraph (Grass Instrument Company, Quincy, Massachusetts). Hepes-buffered solution at 36.5° to 37.0° C and pH 7.40, bubbled with 100% oxygen, flowed continuously through the chamber at 1.3 ml/min.

After spontaneous activity became regular, the strips were exposed to Hepes-buffered solution containing oxytocin (Sigma Chemical Co., St. Louis, Missouri), 800 mU/L, to simulate labor. Strips were then exposed to solutions containing oxytocin and either aminophylline or nifedipine. Three concentrations of aminophylline (40, 100, and 400 μmol/L) and nifedipine (Pfizer Laboratories, New York, New York) (5, 10, and 20 μg/L) were tested. Five to 10 contractions were obtained for each drug solution. Solutions were changed by rapid flushing of at least 60 ml of the new solution through the chamber over a 30-second period. A given strip was exposed to increasing concentrations of either aminophylline or nifedipine, although it may not have been exposed to all three concentrations of the drug.

Solutions. The Hepes-buffered physiologic salt solution contained sodium chloride, 113 mmol/L; potassium chloride, 4.7 mmol/L; magnesium sulfate, 1.2 mmol/L; glucose, 2.8 mmol/L; Hepes, 10 mmol/L; and calcium chloride, 2.5 mmol/L. The pH value was brought to 7.40 with sodium hydroxide. Nifedipine solutions were prepared immediately before an experiment and kept dark until needed.

Data analysis. The period (T) of a contraction was defined as the time interval between the beginning of the contraction of interest and the beginning of the previous contraction. The area under the peak for each contraction is a quantitative measure of the time-integrated force (F). These areas were obtained by cutting out photocopies of the peaks and comparing their weights with those of known standard curves. The average force (G) exerted during the time interval between the contraction of interest and the previous contraction was defined as F/T. (See Comment section for further explanation.)

The values of T, F, and G from individual contractions obtained under the same drug conditions were averaged for each strip. For example, T_{oxytocin} represents the average T from all contractions obtained when a strip was exposed to oxytocin alone (baseline conditions). $T_{\text{aminophylline}}$ represents the average T from all contractions obtained when the strip was exposed to oxytocin plus aminophylline. The response (R) to the drug tested is then defined as the average T, F, or G during drug exposure divided by that parameter during oxytocin stimulation alone (for instance, $R = T_{\text{aminophylline}}/T_{\text{oxytocin}}$). Each myometrial strip thus serves as its own control, and drug effects are expressed as a fraction of the oxytocin baseline parameters.

The Wilcoxon signed-rank test was used to detect statistically significant differences in the responses of T, F and G to drug treatment and to detect whether there was a dose-related effect.

Results

To be certain that the effects on T and F were truly drug effects and not simply smooth muscle fatigue or

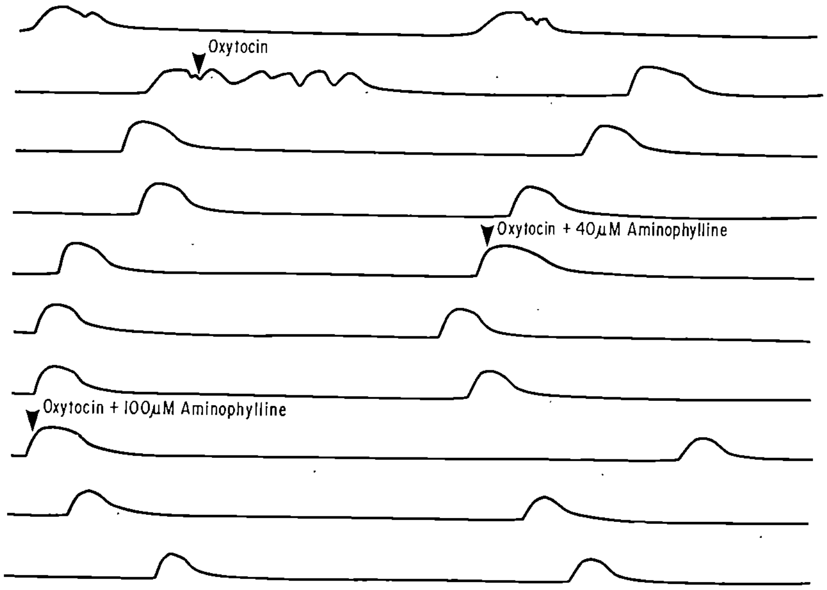

Fig. 2. Inhibition of in vitro myometrial activity by aminophylline.

cell death, six control strips were exposed to oxytocin (800 mU/L) only and allowed to contract for at least 40 contractions, the maximum number of contractions obtained during an experiment. The first 10 contractions after the addition of oxytocin were averaged for the baseline. Fig. 1 shows that strip function, measured by G, was preserved for the duration of the experiments. Similarly, T and F were stable for the duration of the experiments (data not shown).

Aminophylline effects. A representative tracing of a strip treated with aminophylline is shown in Fig. 2. The effects of the three concentrations of aminophylline on T, F, and G are shown in Figs. 3 to 5. Aminophylline lengthened the median T significantly at concentrations of 40 and 100 μmol, but there was lack of significance at 400 μmol, perhaps because of the small number of strips exposed to this concentration (Fig. 3). The median slope of the dose-response curves was not significantly different from zero, indicating there was no dose-related effect (that is, T did not lengthen further with increasing doses).

All concentrations of aminophylline lowered the median F significantly below the baseline (Fig. 4). The median slope of the dose-response curves was significantly different from zero ($p < 0.005$), indicating that the effect of aminophylline on F is dose-related (that is, the greater the dose over the range tested, the greater the reduction of F).

All concentrations of aminophylline lowered G significantly below the baseline (Fig. 5). This effect on G was also dose dependent [that is, the median slope of the dose-response curves was significantly different from zero ($p < 0.005$)], with G reduced as the concentration of aminophylline increased.

Nifedipine effects. A representative tracing of a strip treated with nifedipine is shown in Fig. 6. Figs. 7 to 9 show the effects of the three concentrations of nifedipine on T, F, and G. Nifedipine (5 μg/L) significantly prolonged T; at higher concentrations T progressively decreased, although never significantly below baseline (Fig. 7). The median slope of the dose-response curves was significantly different from zero ($p < 0.005$), indicating that the effect of nifedipine on T was dose related.

The median F was reduced significantly below baseline at all concentrations of nifedipine studied (Fig. 8). The median slope of the dose-response curves was significantly different from zero ($p < 0.005$), indicating a dose-dependent effect (that is, the greater the dose of nifedipine over the range studied, the greater the reduction of F).

Despite no effect on T at the higher concentrations of nifedipine, the dramatic effect of nifedipine on F resulted in depression of G below baseline at all concentrations of nifedipine (Fig. 9). This effect was also dose related (the median slope of the dose-response curves was significantly different from zero, $p < 0.005$), with increasing concentrations producing more profound reduction in G over the dose range studied.

Comment

Justification of the model system. This study measured the tocolytic effects of aminophylline and nifedipine by using isolated myometrial strips. This experimental model is an established means of determining the action of hormones and drugs, including potential tocolytic agents, on uterine smooth muscle.[1, 2]

Specimens were obtained from the lower uterine seg-

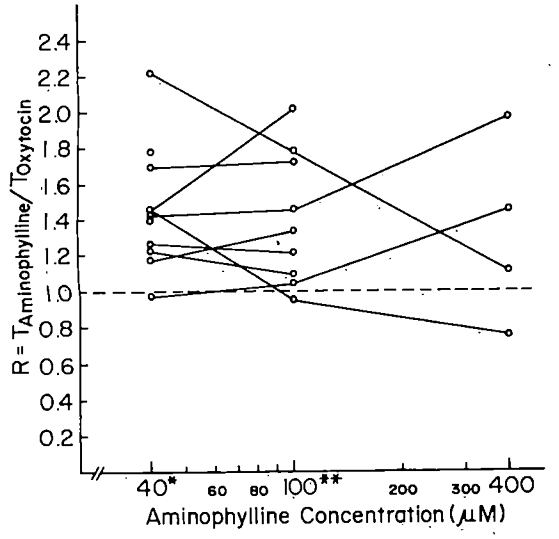

Fig. 3. Effect of aminophylline on the period of contraction of oxytocin-stimulated pregnant human myometrial strips; * = $p < 0.005$; ** = $p < 0.001$.

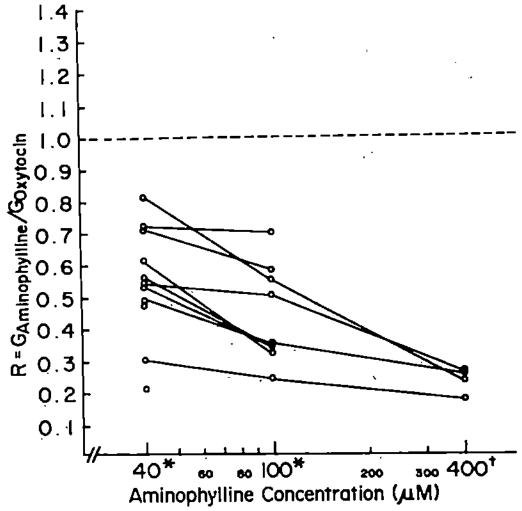

Fig. 5. Effect of aminophylline on the average force of contraction of oxytocin-stimulated pregnant human myometrial strips; * = $p < 0.005$; † = $p < 0.05$.

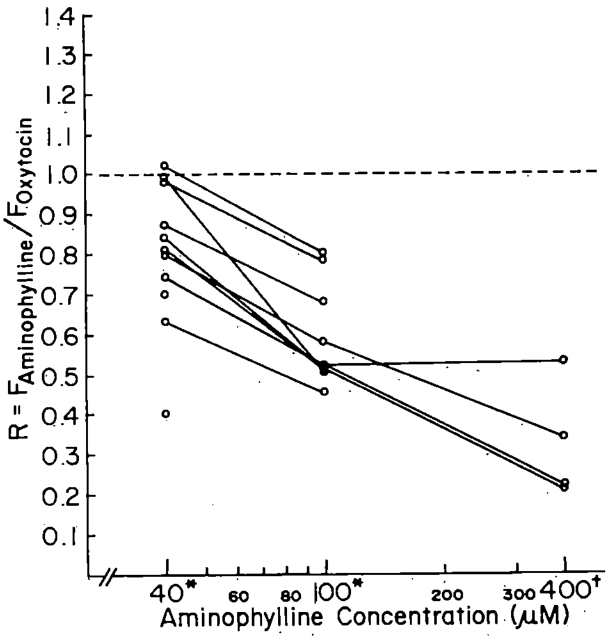

Fig. 4. Effect of aminophylline on the time-integrated force of contraction of oxytocin-stimulated pregnant human myometrial strips; * = $p < 0.005$; † = $p < 0.05$.

ment, which contains relatively large amounts of connective tissue and less smooth muscle compared with the uterine body or fundus. Although the function of the lower uterine segment during parturition is to dilate rather than contract against the fetus, all tissue samples from this area contained smooth muscle, which was spontaneously contractile and responsive to oxytocin. Ideally, the investigation of tocolytic drugs should be conducted with tissues in the clinically relevant range of 26 to 34 weeks' gestation. Because tissue of this age is only infrequently available, these studies were performed on preterm and term specimens.

The goal of tocolytic therapy is to prevent the myometrium from expelling the fetus. The ability of the myometrium to expel the fetus (that is, the effectiveness of labor) is governed by both contraction strength and contraction frequency. Although contraction frequency is easily measured as the inverse of the contraction period (T), contraction strength is less well defined. Neither the peak force that occurs during a contraction nor the contraction duration seems to be an adequate measurement of contraction strength, since clinically, contractions of both sufficient force and duration are necessary to expel the fetus. The act of expelling the fetus requires the myometrium to change the velocity or momentum of the fetus. This ability of the myometrium to change the momentum of the fetus is equal to the impulse that the myometrium can deliver to the fetus. Impulse (dyne-sec) is equal to force (dyne) times the duration (sec) of that force and thus incorporates both elements of contraction strength mentioned above. In our model, impulse is measured by the area under the peak of a contraction (that is, F). This area is an accurate in vitro estimate of contraction strength, since it incorporates both the force and the duration of the contraction.

Because the effectiveness of labor is governed by both contraction strength (F) and contraction frequency (1/T), a third variable, G, was calculated to assess the net effect of both of these parameters. G was defined as F multiplied by the contraction frequency (that is, F divided by T). The Montevideo unit,[3] which

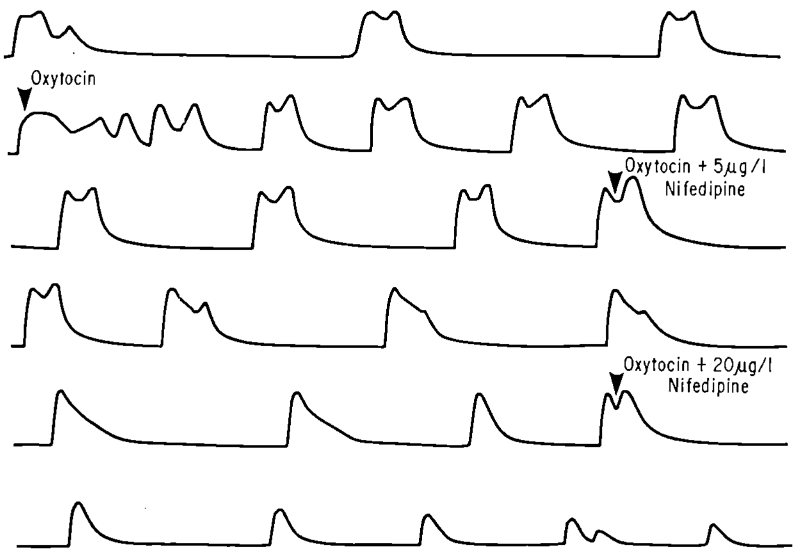

Fig. 6. Inhibition of in vitro myometrial activity by nifedipine.

is used clinically to assess the adequacy of labor, is also a product of contraction strength and contraction frequency. If F in our model is accepted as an adequate in vitro measure of contraction strength, then G becomes an in vitro analogue of the Montevideo unit. Thus G is a measure of the effectiveness of labor.

Although there is good evidence that the concentration of oxytocin receptors, not the amount of circulating oxytocin, is important in the initiation of labor,[4] we stimulated the strips with oxytocin to characterize the effects of aminophylline and nifedepine on strongly contracting tissue. We used a concentration of oxytocin that has been found by others[2] to give about 50% stimulation. In addition to mimicking labor, oxytocin was intended to provide a relative degree of uniformity between tissues, since some were taken from women in active labor and some from nonlaboring women. Exposure to oxytocin always produced at least a transient increase in strip activity (both increased rate and force), a response similar to that seen clinically.

Aminophylline. Aminophylline is a xanthine derivative that has been accepted traditionally as a phosphodiesterase inhibitor. In human myometrial strips we have shown that aminophylline produces a dose-related decrease in F and G and lengthens T in a manner that is not dose related at the concentrations studied. These data indicate that aminophylline decreases contraction strength and contraction frequency and reduces the effectiveness of labor. Therefore aminophylline may be a clinically useful tocolytic agent.

The clinical usefulness of any drug is governed by the balance between its efficacy and toxicity. There have been two clinical trials with aminophylline, which have yielded conflicting results on the favorability of aminophylline's cardiovascular/tocolytic ratio compared with β-sympathomimetic agents.[5, 6] These studies had different end points and used vastly different drug regimens, which may account for their contradictory results. However, aminophylline may prove to be an unsatisfactory tocolytic agent when used alone because of its cardiovascular side effects.

One way in which aminophylline may be particularly useful is in combination with a β-adrenergic receptor agonist. There is a well-known tachyphylaxis that occurs with the use of β-agonists, which may be caused by down regulation of β-adrenergic receptors (short-term) and an increase in phosphodiesterase activity (long-term).[7] The combination of a phosphodiesterase inhibitor such as aminophylline with a β-adrenergic receptor agonist may slow the development of tachyphylaxis as a result of increased phosphodiesterase activity and may provide a synergism between the two classes of drugs.[8] This principle is already applied in asthma therapy, where β-adrenergic receptor agonists and phosphodiesterase inhibitors are frequently combined to reduce side effects and increase efficacy.

Animal work[9, 10] and a clinical trial conducted by Hadjigeorgiou et al.[11] have shown that aminophylline may accelerate fetal lung maturation. This suggests that aminophylline may be able to reduce the risk of respiratory distress syndrome in two ways: first, by accelerating the rate of lung maturation and second, by prolonging pregnancy to allow more time for physiologic lung maturation.

The evidence we present for the tocolytic action of aminophylline combined with the existing data on fetal lung maturation justifies testing aminophylline in controlled, randomized clinical trials for both its tocolytic efficacy and its ability to prevent neonatal respiratory distress syndrome.

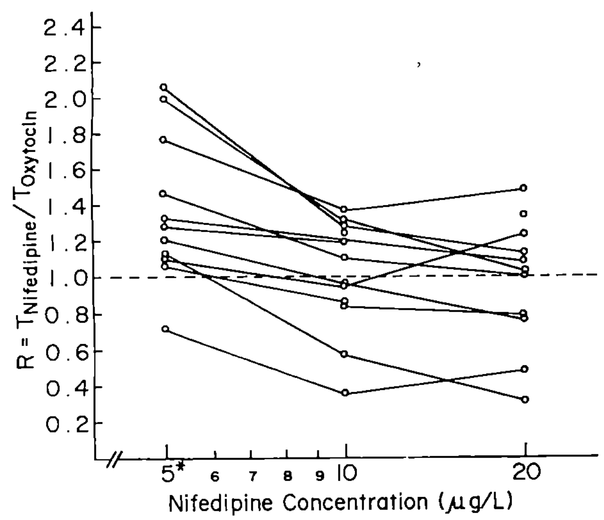

Fig. 7. Effect of nifedipine on the period of contraction of oxytocin-stimulated pregnant human myometrial strips; * = p = 0.005.

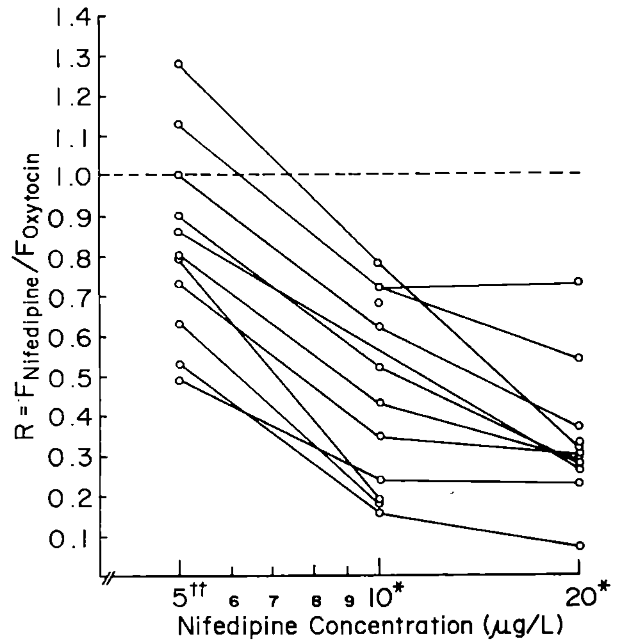

Fig. 8. Effect of nifedipine on time-integrated force of contraction of oxytocin-stimulated pregnant human myometrial strips; †† = $p < 0.05$; * = $p < 0.005$.

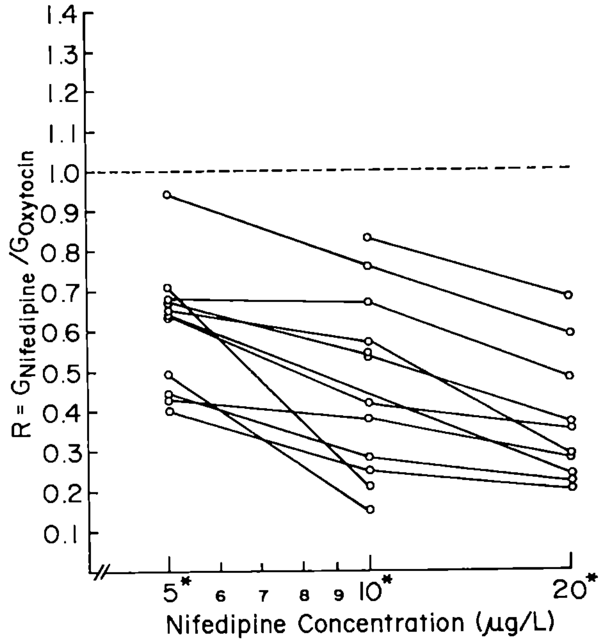

Fig. 9. Effect of nifedipine on average force of contraction of oxytocin-stimulated pregnant human myometrial strips; * = $p < 0.005$.

Nifedipine. Nifedipine is one of a class of drugs that are widely thought to act as blockers of the ionized calcium–mediated slow inward current through antagonism of voltage-sensitive calcium channels in the sarcolemma.[12] We have shown in human myometrial strips that nifedipine produces a dose-related decrease in F and G. There was no consistent increase in T. These results indicate that nifedipine reduces the effectiveness of labor primarily by reducing contraction strength. Perhaps in combination with another drug that reliably reduces the rate of contraction, nifedipine would be a valuable tocolytic agent.

Our results support those of Forman et al.,[1] who found that both pregnant and nonpregnant strips of myometrium responded to nifedipine with decreased amplitude of contraction in a dose-dependent manner. Although we demonstrated an initial lengthening followed by a shortening of T with nifedipine treatment, Forman et al.[1] found only a shortening of T before abolishment of activity. In vivo studies[13] have shown variable results on T (see below). This confusing relationship between nifedipine and T may reflect differences in experimental method but may also reflect the complex contribution of ionized calcium to the slow pacemaker potential and/or resting membrane potential that govern the initiation of contraction.

There have been several clinical trials with nifedipine reported in the European literature.[14-16] Nifedipine inhibited prostaglandin $F_{2\alpha}$–induced uterine contractions in pregnant patients undergoing midtrimester abortion by decreasing the intensity and slightly increasing the frequency of contractions.[14] Nifedipine was also able to suppress oxytocin– and prostaglandin $F_{2\alpha}$–induced activity in the postpartum uterus; this was caused by a decrease in amplitude and frequency.[15]

Ulmsten et al.[16] treated 10 women in premature labor with nifedipine to delay delivery for at least 3 days while glucocorticoids were administered. The side effects noted were a facial flush within 15 minutes of drug administration and a transient rise in maternal pulse (10 to 25 bpm). No significant changes were noted in the fetal heart rate, and there were no neonatal complications. Although this study is encouraging, there have been recent reports of dangerous side effects of calcium channel antagonists in ewes. For example, Golichowski et al.[17] found that nifedipine significantly increased fetal heart rate compared with control and ritodrine treatment. Parisi et al.[18] reported the development of hypercapnia, acidosis, and possibly hypoxemia in fetuses of hypertensive ewes treated with nicardipine, another calcium channel antagonist. More studies are required to elucidate the fetal risks of nifedipine treatment.

A striking finding in our results was the wide variability in tissue response to both aminophylline and nifedipine. Some variability is inherent in working with human tissue, and some is probably attributable to the wide range of gestational ages. The variability in response to aminophylline may be caused by differences in turnover rates of cyclic adenosine monophosphate. The variability in response to both drugs may be caused by differing amounts of connective tissue between myometrial strips, resulting in unequal diffusion of the drugs into smooth muscle cells. The degree of variability in these in vitro experiments may indicate that patient variability will also be large.

The number of samples was too small to permit statistical comparison of subgroups such as term and preterm, laboring and nonlaboring, or administration of general and regional anesthetics. However, no obvious trends were seen with regard to these clinical parameters.

In summary, we have demonstrated that both aminophylline and nifedipine may be useful tocolytic agents. Further studies to investigate their precise mechanisms of action may indicate which clinical settings are most appropriate for each drug. Future animal work should clarify the maternal and fetal dangers of nifedipine treatment and may indicate whether this drug can be safely tested clinically. Convincing evidence of the tocolytic ability of aminophylline has been presented here and by others.[5,6,8] Both in vitro and clinical studies of these drugs alone or in combination with other drugs should be the next undertaking.

We thank Deborah Dawson for her assistance in the statistical analysis of these data.

REFERENCES

1. Forman A, Andersson KE, Persson CG, Ulmsten U. Relaxant effects of nifedipine on isolated, human myometrium. Acta Pharmacol Toxicol 1979;45:81.
2. Wikland M, Lindblom B, Wilhelmsson L, Wiqvist N. Oxytocin, prostaglandins, and contractility of the human uterus at term pregnancy. Acta Obstet Gynecol Scand 1982;61:467.
3. Caldeyro-Barcia R, Sica-Blanco Y, Poseiro J, et al. A quantitative study of the action of synthetic oxytocin on the pregnant human uterus. J Pharmacol Exp Ther 1957; 121:18.
4. Fuchs A, Fuchs F, Husslein P, Soloff M, Fernstrom M. Oxytocin receptors and human parturition: a dual role for oxytocin in the initiation of labor. Science 1982; 215:1396.
5. Lipshitz J. Uterine and cardiovascular effects of aminophylline. AM J OBSTET GYNECOL 1978;131:716.
6. Melis G, Fruzzetti F, Strigini F, et al. Aminophylline treatment of preterm labor. Acta Eur Fertil 1984;15:357.
7. Berg G, Andersson R, Ryden G. Effects of selective beta-adrenergic agonists on spontaneous contractions, cAMP levels and phosphodiesterase activity in myometrial strips from pregnant women treated with terbutaline. Gynecol Obstet Invest 1982;14:56.
8. Berg G, Andersson R, Ryden G. In vitro study of phosphodiesterase-inhibiting drugs: a complement to beta-sympathomimetic drug therapy in premature labor? AM J OBSTET GYNECOL 1983;145:802.
9. Karotkin E, Kido M, Cashore W, et al. Acceleration of fetal lung maturation by aminophylline in pregnant rabbits. Pediatr Res 1976;10:722.
10. Barrett C, Sevanian A, Phelps D, Gilden C, Kaplan S. Effects of cortisol and aminophyllin upon survival, pulmonary mechanics, and secreted phosphatidyl choline of prematurely delivered rabbits. Pediatr Res 1978;12:38.
11. Hadjigeorgiou E, Kitsiou S, Psaroudakis A, Segos C, Nicolopoulos D, Kaskarelis D. Antepartum aminophylline treatment for prevention of the respiratory distress syndrome in premature infants. AM J OBSTET GYNECOL 1979;135:257.
12. Fleckenstein A. Specific pharmacology of calcium in myocardium, cardiac pacemakers, and vascular smooth muscle. Annu Rev Pharmacol Toxicol 1977;17:149.
13. Anderson N. Physiologic basis of myometrial function. Semin Perinatol 1978;2:211.
14. Andersson K, Ingemarsson I, Ulmsten U, Wingerup L. Inhibition of prostaglandin-induced uterine activity by nifedipine. Br J Obstet Gynaecol 1979;86:175.
15. Forman A, Gandrup P, Andersson K, Ulmsten U. Effects of nifedipine on oxytocin- and prostaglandin $F_{2\alpha}$-induced activity in the postpartum uterus. AM J OBSTET GYNECOL 1982;144:665.
16. Ulmsten U, Andersson K, Wingerup L. Treatment of premature labor with the calcium antagonist nifedipine. Arch Gynecol 1980;229:1.
17. Golichowski A, Hathaway D, Fineberg N, Peleg D. Tocolytic and hemodynamic effects of nifedipine in the ewe. AM J OBSTET GYNECOL 1985;151:1134.
18. Parisi V, Salinas J, Stockmar E. Fetal cardiorespiratory responses to maternal administration of nicardipine in the hypertensive ewe [Abstract]. In: Proceedings of the sixth annual meeting of The Society of Perinatal Obstetricians, San Antonio, Texas, January 30–February 1, 1986.

Factor XI deficiency presenting as hemarthrosis during pregnancy

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This report describes events and findings in a pregnant patient with factor XI deficiency who suffered at least one episode of hemarthrosis. The incidence and rarity of this complication are discussed and views on management expressed. (AM J OBSTET GYNECOL 1987;157:178-9.)

Key words: Factor XI deficiency, hemarthrosis, pregnancy

Deficiency of coagulation factor XI is an autosomal recessive or partially dominant inherited coagulopathy. Many patients with this disorder go through life unaware of their condition until challenged by surgery or trauma. The incidence of hemarthrosis in factor XI–deficient patients is very rare.[1] Coagulation factors XI and XIII are known to decrease during pregnancy.[2, 3] This article reports a previously silent case of factor XI deficiency in a woman who developed hemarthrosis late in pregnancy.

Case report

A 26-year-old legal secretary (gravida 2, para 1, abortions, 0) of Ashkenazi Jewish ancestry was seen at 37 weeks gestation with atraumatic spontaneous pain and swelling of the left knee. On aspiration of the knee, 18 ml sterile blood was obtained. She denied easy bruising, and extractions of three wisdom teeth were uneventful. There was no family history of a hemorrhagic diathesis. Her first pregnancy and delivery with an epidural anesthetic had been uncomplicated apart from an uninvestigated swollen right knee during the ninth month, blood loss of 250 ml during delivery, and slightly increased bleeding during the first night.

The initial prothrombin time was 9.5 seconds (control 10 seconds), the activated partial thromboplastin time was 50 seconds (control 34 seconds), Ivy bleeding time was 9 minutes (normal 5 to 10 minutes), and platelet count was 255,000/mm^3. Fibrinogen was 2.6 gm/L. Factor VIII assay was 100%. The factor XI one-stage activated partial thromboplastin time activity assay was 7% of normal but rose to 28% with fresh–frozen plasma infusion. This assay is based on the ability of the patient's plasma to correct the prolonged activated partial thromboplastin time of known factor XI–deficient plasma. (Normal range for women in the third trimester is 51% to 89% of normal.)[3] She was delivered uneventfully under inhalation anesthesia after receiving fresh-frozen plasma. At 1½, 8, and 18 months post partum, factor XI activity assays were 6%, 9%, and 8% of normal, respectively. A third pregnancy terminated in spontaneous abortion. When she was 3, 7, and 8 months into her fourth pregnancy, the factor XI assays were 14%, 6%, and 8% of normal, respectively. Immediately before this delivery, factor XI was 4% but rose to 19% with fresh-frozen plasma. The patient remains symptom free 6 years after this fourth pregnancy.

Comment

Spontaneous hemarthrosis in patients with factor XI deficiency is very rare, and the presence or absence of bleeding symptoms seems to be consistent within a factor XI–deficient kindred rather than related only to the individual patient's factor XI level.[1, 4] Factor XI deficiency is most commonly found in Ashkenazi Jews and symptomatic bleeding is found equally distributed between homozygous and heterozygous individuals.[1, 4, 5]

Our patient's parents were free of symptoms and were never tested for factor XI activity, but her repeated factor XI assays of <15% would suggest that she is homozygous for the deficiency.[5] However, she, like her parents, never had any hemorrhagic tendency until the episodes of hemarthrosis that occurred during pregnancy. The degree of postpartum bleeding after her first delivery was not sufficient to suggest a coagulopathy, and it did not delay her discharge from the hospital or trigger laboratory investigation.

Factor XI assays and bleeding tendency in this disorder do not correlate well; therefore it is difficult to assign a hemostatic level in factor XI–deficient patients before surgery or delivery.[1, 4] Consequently, serial laboratory tests fail to aid in assessing intelligently the amount of fresh-frozen plasma needed to ensure hemostasis.

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In our series of 21 factor XI–deficient women, 17 have been delivered of 41 children while their factor XI status was unknown. Only six of these deliveries (one of which involved twins) were alleged by the patient to be complicated by excessive bleeding. No blood transfusions were known to be given. Three patients had normal childbirth but bled with either tooth extraction or surgical procedures.

Phillips et al.[3] have demonstrated a gradual but statistically significant fall in factor XI during the second and third trimester compared with the level in nonpregnant women. In the third trimester the range for factor XI was 51% to 89% whereas the normal nonpregnant range was 83% to 133%.[3] This reduction in factor XI is not such that bleeding would be expected. Our patient was shown to have similar factor XI levels during and outside pregnancy. The reason for factors XI and XIII to fall in pregnancy while other coagulation factors rise is not clear. Phillips et al. speculate that it may be a protective mechanism to decrease antepartum thrombotic episodes.[3]

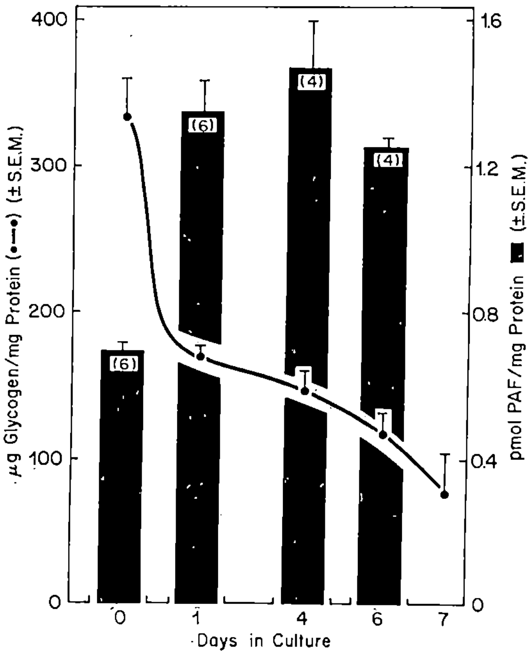
In summary, a silent case of factor XI deficiency presented with hemarthrosis late in pregnancy. Uneventful delivery occurred after infusion of fresh-frozen plasma. Factor XI supplement was advised because of the spontaneous hemarthrosis near term. In our experience women with factor XI deficiency tolerate childbirth without catastrophic hemorrhage and will not need fresh-frozen plasma; however, fresh-frozen plasma may be warranted for pregnant women with factor XI deficiency and a bleeding history or indications of obstetric complications.

REFERENCES

1. Biggs R, Rizza CR. Human blood coagulation, hemostasis and thrombosis, ed 3. Oxford: Blackwell Scientific, 1984: 149;259-60; 299-300.
2. Bonnar J. Acute and chronic coagulation problems in pregnancy. In: Poller L, ed. Recent advances in blood coagulation, No 2. New York: Churchill Livingstone, 1977:363.
3. Phillips LL, Rosano L, Skrodelis V. Changes in factor XI (plasma thromboplastin antecedent) levels during pregnancy. Am J Obstet Gynecol 1973;116:1114-6.
4. Ragni MV, Sinka D, Seaman F, Lewis JH, Spero JA, Walsh PN. Comparison of bleeding tendency, factor XI coagulant activity and factor XI antigen in twenty-five factor XI-deficient kindreds. Blood 1985;65:719-24.
5. Saito H, Ratnoff OD, Bouma BN, Seligsohn U. Failure to detect variant (CRM) plasma thromboplastin antecedent (factor XI) molecules in hereditary plasma thromboplastin antecedent deficiency: a study of 125 patients of several ethnic backgrounds. J Lab Clin Med 1985;106:718-22.

Correction

In the article by Hoffman et al., entitled "The role of placelet-activating factor in human fetal lung maturation" (Am J Obstet Gynecol 1986;155:70-5), in Fig. 4, on page 73, the axis was mislabeled. The correct Fig. 4 is shown here.



Current Development

Hydatidiform mole: Cytogenetically unusual cases and their implications for the present classification

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Unusual observations in hydatidiform moles are of clinical importance as well as of theoretical interest when the present subgrouping into partial or complete moles is evaluated, particularly in view of the more malignant potential of the latter. The purpose of the present review is to summarize, update, and evaluate information on moles with unusual karyotypes or origins. The important factor in the evolution of partial or complete moles appears to be the ratio of maternal to paternal chromosomes and not the ploidy of the tissue. Complete hydatidiform moles develop from androgenetic conceptions while partial hydatidiform moles develop from those conceptions in which the number of paternal sets of chromosomes exceeds the number of maternally derived sets. Rare cases inconsistent with this concept emphasize the value of examining morphologic features, cell ploidy, and genetic markers when one is attempting to classify hydatidiform moles. (Am J Obstet Gynecol 1987;157:180-4.)

Key words: Hydatidiform mole, classification, chromosome analysis, cytogenetically unusual cases

Hydatidiform moles are separated into at least two distinct entities on the basis of morphologic and cytogenetic characteristics.[1-3] Complete hydatidiform mole has widespread cystic swelling of the villi, it lacks the presence of fetal elements, and the chromosomal origin is by diploid androgenesis, that is, all 46 chromosomes are paternally derived.[4-11] Partial hydatidiform mole has villous morphologic features ranging from normal villi to pronounced cystic dilatation, is accompanied by a fetus, or presents evidence of the previous existence of a fetus by the presence of erythroblasts.[12] The chromosomal constitution is generally triploid with the extra chromosomal complement of paternal origin.[13, 14]

Continuing research on the cytogenetics of hydatidiform mole in an attempt to elucidate possible cytogenetic markers of increased malignant potential has revealed occasional numeric aberrations indicating a more complex genetic background of hydatidiform mole than outlined above. Reports on rare diploid, partial hydatidiform mole[3, 15] and complete hydatidiform mole comprising genetic markers consistent with a normal conception[10, 16] support suggestions of more than two subgroups.

The purpose of the present review is to summarize and evaluate information on unusual moles cited in the literature and observed by us, which deviate cytogenetically from the concepts of paternally derived triploid partial hydatidiform mole and diploid complete hydatidiform mole of androgenetic origin. Combining the experiences of four institutions with a well-established interest in the genetic background of hydatidiform mole, we hope to shed further light on the significance of outstanding cases on the present classification of hydatidiform mole.

Tetraploid hydatidiform moles

Cytogenetic studies of molar tissue are usually made on cultured cells. Such studies have revealed some cases in which the molar tissue was tetraploid (Table I). Makino et al.[19] have previously described tetraploid cells in direct preparations of molar tissue, although they

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Table I. Tetraploid hydatidiform moles

Series	*Year*	*Karyotype*	*Origin*	*Morphologic appearance*	*Markers examined*
Jacobs et al.[6]	1978	85,XXXX	p,p,p,p	Complete	Q
Sheppard et al.[17]	1982	92,XXXX	p,p,p,m	Partial (?)	Q, C, l.a., enz.
Surti et al.[18]	1986	92,XXXY	p,p,p,m	Partial	Q
		92,XXXY	p,p,p,m	Partial	Q
Vejerslev et al.[18a]	1987	91,XXXX, −4	p,p,p,m	Complete	Q, AgNOR

p = Paternal haploid set; m = maternal haploid set; Q = quinacrine banding; C = C banding; l.a. = lateral asymmetry; enz. = isoenzymes; AgNOR = silver staining.

found that tetraploid cells occurred rarely in normal villi. In 1978 Jacobs et al.[6] reported a 92,XXXX tetraploid complete mole with the major mode at 85 to 86 and a minor cell line in the 55 to 57 range. Chromosome polymorphisms showed all chromosomes to be paternally derived and the karyotype observed was probably superimposed on a basic 46,XX pattern.

True tetraploidy has been observed in three molar specimens.[18, 18a] All three cases had chromosomal markers indicating one maternal and three paternal contributions. The mechanisms involved in the origin of these cases are most likely fertilization of a normal, haploid ovum by three sperm or by two sperm, one of which is diploid. Two cases had morphologic features of a partial hydatidiform mole[18] and one had morphologic characteristics resembling a complete hydatidiform mole.[18a] One case with a similar genetic constitution has been described by Sheppard et al.[17] Macroscopically this specimen appeared to be a partial mole but on microscopic investigation there was no trophoblastic hyperplasia, considered to be an essential feature for a diagnosis of hydatidiform mole. However, trophoblastic hyperplasia in partial moles is focal and during the first trimester it is often indistinguishable from physiologic hyperplasia. Thus the diagnosis of partial mole cannot be *excluded* in this case.

Triploid hydatidiform moles

Moles that are triploid are generally partial and comprise two paternal and one maternal set of chromosomes. However, two types of exception to this have been described.

Triploid androgenesis. To our knowledge only two cases of triploid androgenesis have been observed[18a] (Surti U, unpublished observations). One had a 69,XXY karyotype with all chromosomes of paternal origin according to Q markers. In the second the karyotype was 70,XXY,+7 and the paternal origin was indicated by chromosomal heteromorphisms after QM banding and AgNOR staining. Both were morphologically complete moles, which is consistent with an androgenetic origin of the chromosome complements.

Maternally derived triploids. Three maternally derived triploids in 54 cases of morphologically partial moles were initially described by Jacobs et al.[14] On a recent revision these specimens were reclassified as hydropic degeneration in spontaneous abortion (Szulman AE, personal communication).

Diploid hydatidiform moles

Complete diploid moles have been found to have entirely paternal chromosomes, showing a 46,XX[4-11] or 46,XY chromosome constitution.[9, 20-24] The origin of homozygous 46,XX moles is most likely the consequence of the fertilization of an "anucleate" egg by a haploid sperm with subsequent doubling of the chromosome number.[7, 8, 10, 11] A few cases (3% to 13%) of androgenetic diploid moles show heterozygosity indicating fertilization by dispermy. These may be 46,XY[9, 22-24] or 46,XX moles.[25, 26] Well-defined series on cytogenetics in molar pregnancies show a close relation between diploid androgenesis and morphologically complete moles. However, some exceptions have been described in the literature or observed by the authors.

Cytogenetically normal conceptions (Table II). Complete moles having genetic markers consistent with a normal conception present an intriguing problem. The first case was reported by Jacobs et al.[10] in 1980. The authors subsequently reported that this specimen fell morphologically into a separate category.[27] Nucleated erythrocytes were present in the placental capillaries, indicating the existence of a fetomaternal circulation, which is not in accordance with the pathologic features of a complete mole.

Vejerslev et al.[28a] found a complete, heterozygous 46,XY mole showing genetic markers compatible with a normal conception. A maternal and a paternal contribution to the genome was observed both by chromosome marker analysis and by HLA typing. Davis et al.[16] cultured molar tissue from eight cases for cytogenetic investigation. Four were suitable for chromosome marker analysis, and of these the two informative cases were consistent with a normal conception.

As evidenced by the difficulty of culturing tissue from complete moles, as reported in larger series,[3, 11, 13, 28, 29] a "normal" cell line may be more adaptable to in vitro culture conditions than a cell line originating from dip-

Table II. Diploid hydatidiform moles with maternal and paternal contributions

Series	*Year*	*Karyotype*	*Origin*	*Morphologic appearance*	*Markers examined*
Jacobs et al.[10]	1980	46,XX	p,m	Complete	Q, C, PGM$_1$
Davis et al.[16]	1984	46,XX	p,m	Hydatidiform mole	Q
		46,XY	p,m	Hydatidiform mole	Q
Vejerslev et al.[28a]	1987	46,XY	p,m	Complete	Q, C, AgNOR, PGM$_1$, ACP$_1$, EsD, HLA

p = Paternal haploid set; m = maternal haploid set; Q = quinacrine banding; C = C banding; PGM$_1$ = phosphoglucomutase; AgNOR = silver staining; ACP$_1$ = acid phosphatase; EsD = esterase D; HLA = tissue antigens.

loid androgenesis. Thus the observation of chromosome polymorphisms exclusively consistent with normal gestation may indicate a twin gestation in which only the normal cell line was identified, the androgenetic cell line failing to grow in culture. This is less likely in the case of Vejerslev et al.[28a] where genetic heteromorphisms consistent with a normal conception were observed in both cultured and uncultured material.

Diploid partial moles. In a series of 23 molar specimens investigated cytogenetically and morphologically Szulman and Surti[3] described diploidy in 11 complete moles and triploidy in 11 partial moles. However, in one morphologically partial mole with a diploid karyotype, in contrast to the triploidy expected, trophoblastic inclusions and scalloping of the villi were absent. The possibility of maternal contamination was ruled out. The origin of the chromosome complements was not available. The authors considered the possibility of a third group comprising diploid partial moles.

To our knowledge morphologically partial moles have not been described in association with diploidy arising by androgenesis. Chromosome polymorphisms were not investigated in the case of Szulman and Surti[3] or in the three cases of diploid partial moles described by Teng and Ballon.[15] This raises some other possibilities for the observation of diploid partial moles besides a third subgroup. The 46,XX karyotype would be expected in all cultures in which molar cells are outgrown by contaminating maternal cells. This has been observed during extended in vitro culture of molar tissue (Fisher RA, Vejerslev LO, et al., unpublished observations). Again twin gestation in which only the normal line, and not the molar line, was identified in culture could be an explanation for these results. Without the benefit of full analysis of chromosomal polymorphisms it is not possible to establish a causal relationship between the karyotype and the morphologic changes observed.

Twin gestation (Table III). Conceptions comprising diploid androgenesis and a normal conception as evidenced by chromosomal markers have been reported in seven cases[30-33] ((Table III). In five cases a fetus was present and twin pregnancy was observed by the study of polymorphisms in both fetal and placental tissue. In one case a normal 46,XY chromosome constitution was found in a culture from a homogeneous complete mole from which a homozygous, androgenetic 46,XX cell line was also derived.[33] These cases require special attention because of the possibility of a misdiagnosis of a partial mole instead of a complete mole accompanied by a normal twin.

Hypoploid or hyperploid moles

These are occasionally observed in larger series comprising cytogenetic investigations of hydatidiform moles.[10, 13, 14, 18a] The information obtained by chromosomal marker analysis indicates that an extra, paternally derived chromosome complement is the major feature correlated with hydatidiform mole irrespective of single chromosome aberrations. No single chromosome was preferentially involved. Assuming that the supernumerary paternal chromosome set is of major importance to hydatidiform mole, the presence of an extra chromosome of maternal origin would be of special interest. In a paternally derived triploid mole having an extra chromosome 21, this chromosome was of maternal origin arising from a failure at the first meiotic division. Morphologically the characteristics of a partial mole were present.[18a] Thus hypotriploidy or hypertriploidy having two paternal and one maternal chromosome complements could conveniently be regarded as triploid partial moles not deserving a special cytogenetic status at present.

In diploid androgenetic complete moles having a supernumerary chromosome, neither outstanding morphologic features nor extraordinary malignant sequelae have been reported.[27] Trisomy in association with one maternal and one paternal chromosome sets appears cytogenetically on the borderline, closer to nonmolar abortion than to mole. Morphologically, however, some of these do fulfill the criteria for partial mole (Szulman AE, personal communication; Wake N, unpublished observations).

Monosomy X has been reported in a complete mole by Berkowitz et al.[34] and in a partial mole by Jacobs et al.[27] However, either cytogenetic marker analysis was inconclusive or the possibility of mislabeling of the spec-

Table III. Twin gestations: Cytogenetic markers indicating diploid androgenesis and normal gestation

		Normal conceptus		*Molar tissue*		
Series	*Year*	*Karyotype*	*Origin*	*Karyotype*	*Origin*	*Markers examined*
Honda et al.[30]	1980	46,XY	m,p	46,XX	p,p	HLA
Fisher et al.[31]	1982	46,XY	m,p	46,XX	p,p	Q, G, C
Ohama et al.[32]	1985	46,XY	m,p	46,XX	p,p	PGM_1, EsD
		46,XX	m,p	46,XX	p,p	$PGM_{1,3}$, EsD
Vejerslev et al.[33]	1986	46,XX	m,p	46,XX	p,p	Q, AgNOR
		46,XY	m,p	46,XX	p,p	Q, AgNOR
Surti (unpublished observations)	1986	46,XX	m,p	46,XX	p,p	Q

m = Maternal haploid set; p = paternal haploid set; HLA = tissue antigens; Q = quinacrine banding; G = G banding; C = C banding; PGM_1 = phosphoglucomutase; EsD = esterase D; AgNOR = silver staining.

imen was noted. Alternatively these cases may represent the loss or gain of a chromosome in diploid, androgenetically derived complete moles. This could have been an in vitro or an in vivo phenomenon.

Comment

Occasional reports of unusual observations generally have little impact on nomenclature on the basis of results of larger series. However, it is of clinical importance as well as of theoretical interest to evaluate the correlation between atypical moles and the present subgrouping into partial and complete moles, particularly in view of the more malignant potential of the latter. With increasing experience in general and information on more cases of aberrant hydatidiform moles, some intriguing observations seem to be more readily explained.

Tetraploid conceptuses with duplication of both maternal and paternal chromosomes indicating a failure at the first cleavage division of the zygote are occasionally observed in nonmolar abortions, whereas three paternal and one maternal chromosome sets appear to be associated with morphologic features resembling partial moles.

The presence of three paternal contributions could arise by several different mechanisms, the most likely being fertilization of an egg by two spermatozoa, one haploid and one diploid, because of failure of the first or second meiotic division or fertilization of an egg by three spermatozoa. In the two cases in which a Y chromosome was present, all three alternatives are possible, whereas the absence of a Y chromosome in two cases excluded a diploid spermatozoon with failure at the first meiotic division. In these cases a conclusive diagnosis cannot be obtained by chromosome analysis. However, examination of sufficient markers makes it possible to determine which is statistically more likely, as in the case of Sheppard et al.[17] where the results were in favor of an origin by trispermy.

Chromosome polymorphisms indicating a normal diploid conception in morphologically partial or complete molar placentas are essentially consistent with twinning. Thus "diploid, partial" or "normal conception, complete" moles do not necessarily imply further division of the concept of hydatidiform mole.

Hydatidiform mole can still be classified into two groups, partial or complete. The former is generally triploid with two paternal sets of chromosomes but may be characterized by tetraploidy with three sets of chromosomes of paternal origin. Complete moles are generally diploid and androgenetic but may be characterized by triploidy or tetraploidy where all chromosome complements are paternally derived. As recently proposed by Surti et al.[18] the important factor appears to be the ratio of paternal to maternal chromosomes and not the ploidy of the tissue. An equal contribution to the genome by both parents results in a normal conception, and a maternal contribution to the genome with excess paternal contributions leads to the development of a partial mole, while a purely androgenetic conceptus develops the characteristics of a complete mole.

A few cases, such as the tetraploid mole with maternal contribution but complete molar morphologic features, do not fit these criteria. Since partial moles combine features of a normal conception and those of a complete mole,[35] it can sometimes be difficult to distinguish pathologically between a partial mole and a complete mole or in some cases between a partial mole and a nonmolar conceptus, where adequate sampling of the tissue is not possible. This may account for the "nonmolar" histologic diagnosis of the tetraploid case that was macroscopically and genetically a partial mole.

The cytogenetically normal chromosome complements of a homogeneous complete molar mass without evidence of a previous fetus do not readily fit the theory that the ratio of maternal : paternal chromosomes is decisive in morphologic appearance. However, the theory proposed by Kajii and Ohama[4] that doubling of a recessive mutation accounted for the propensity of hydatidiform moles to become *malignant* could theoretically also be used to explain the *development* of molar conceptuses. Rare cases of genetically normal conceptions developing as complete moles would then be expected. In these cases the possibility of the legal father not being the biologic father should also be considered and evaluated by application of as many heteromorphisms as available.

An important aspect of the unusual cases is that mere statement of ploidy or number of Bar bodies is of limited value in the cytogenetic characterization of a molar specimen. Chromosome marker analysis seems to be an important tool, confirming pathologic diagnosis and yielding information on characteristics related to the malignant potential, which is not always obtainable by macroscopic or microscopic inspection. Markers consistent with a normal conception in homogeneous complete molar tissue emphasize that cytogenetics and detailed morphologic appearance are complementary in the evaluation of contradictory cases and of less informative value when considered in isolation.

REFERENCES

1. Vassilakos P, Kajii T. Hydatidiform mole: two entities. Lancet 1976;1:259.
2. Vassilakos P, Riotton G, Kajii T. Hydatidiform mole: two entities. A morphologic and cytogenetic study with some clinical considerations. AM J OBSTET GYNECOL 1977;127:167-70.
3. Szulman AE, Surti U. The syndromes of hydatidiform mole. I. Cytogenetic and morphologic correlations. AM J OBSTET GYNECOL 1978;131:665-71.
4. Kajii T, Ohama K. Androgenetic origin of hydatidiform mole. Nature 1977;268:633-4.
5. Wake N, Takagi N, Sasaki M. Androgenesis as a cause of hydatidiform mole. JNCI 1978;60:51-7.
6. Jacobs PA, Hassold TJ, Matsuyama AM, Newlands IM. Chromosome constitution of gestational trophoblastic disease. Lancet 1978;2:49.
7. Lawler SD, Pickthall VJ, Fisher RA, Povey S, Evans MW, Szulman AE. Genetic studies of complete and partial hydatidiform moles. Lancet 1979;2:580.
8. Yamashita K, Wake N, Araki T, Ichinoe K, Makoto K. Human lymphocyte antigen expression in hydatidiform mole: androgenesis following fertilization by a haploid sperm. AM J OBSTET GYNECOL 1979;135:597-600.
9. Vejerslev LO, Mogensen B, Olsen S. Hydatidiform mole in a Danish material. Morphology and cytogenetics. A preliminary communication. Acta Radiol Oncol 1984;23:97-102.
10. Jacobs PA, Wilson CM, Sprenkle JA, Rosenhein NB, Migeon BR. Mechanism of origin of complete hydatidiform moles. Nature 1980;286:714-6.
11. Lawler SD, Povey S, Fisher RA, Pickthall VJ. Genetic studies on hydatidiform moles. II. The origin of complete moles. Ann Hum Genet 1982;46:209-22.
12. Szulman AE, Philippe E, Boué JG, Boué A. Human triploidy: association with partial hydatidiform moles and nonmolar conceptuses. Hum Pathol 1981;12:1016-21.
13. Lawler SD, Fisher RA. Genetic studies on hydatidiform moles. I. The origin of partial moles. Cancer Genet Cytogenet 1982;5:309-20.
14. Jacobs PA, Szulman AE, Funkhouser J, Matsuura JS, Wilson CC. Human triploidy: relationship between parental origin of the additional haploid complement and development of partial hydatidiform mole. Ann Hum Genet 1982;46:223-31.
15. Teng NNH, Ballon SC. Partial hydatidiform mole with diploid karyotype: report of three cases. AM J OBSTET GYNECOL 1984;150:961-4.
16. Davis RJ, Surwit EA, Garay JP, Fortier KJ. Sex assignment in gestational trophoblastic neoplasia. AM J OBSTET GYNECOL 1984;148:722-5.
17. Sheppard DM, Fisher RA, Lawler SD, Povey S. Tetraploid conceptus with three paternal contributions. Hum Genet 1982;62:371-4.
18. Surti U, Szulman AE, Wagner K, Leppert M, O'Brien SJ. Tetraploid partial hydatidiform moles: two cases with a triple paternal contribution and a 92,XXXY karyotype. Hum Genet 1986;72:15-21.
18a. Vejerslev LO, Dissing J, Hansen HE, Poulsen H. Hydatidiform mole: genetic origin in polyploid conceptuses. Hum Genet 1987;76:11-9.
19. Makino S, Sasaki MS, Fukushima T. Preliminary notes on the chromosomes of human chorionic lesions. Proc Acad Jpn 1963;39:54-8.
20. Surti U, Szulman AE, O'Brien S. Complete (classic) hydatidiform mole with 46,XY karyotype of paternal origin. Hum Genet 1979;51:153-5.
21. Ohama K, Kajii T, Okamoto E, et al. Dispermic origin of XY hydatidiform moles. Nature 1981;292:551-2.
22. Pattillo RA, Sasaki S, Katayama KP, Roesler M, Mattingly RF. Genesis of 46,XY hydatidiform mole. AM J OBSTET GYNECOL 1981;141:104-5.
23. Surti U, Szulman AE, O'Brien S. Dispermic origin and clinical outcome of three complete hydatidiform moles with 46,XY karyotype. AM J OBSTET GYNECOL 1982;144:84-7.
24. Fisher RA, Sheppard DM, Lawler SD. Two patients with complete hydatidiform mole with 46,XY karyotype. Br J Obstet Gynaecol 1984;91:690-3.
25. Fisher RA, Lawler SD. Heterozygous complete hydatidiform moles: do they have a worse prognosis than homozygous complete moles? Lancet 1984;1:51.
26. Wake N, Seki T, Fujita H, et al. Malignant potential of homozygous and heterozygous complete moles. Cancer Res 1984;44:1226-30.
27. Jacobs PA, Hunt PA, Matsuura JS, Wilson CC. Complete and partial hydatidiform mole in Hawaii: cytogenetics, morphology and epidemiology. Br J Obstet Gynaecol 1982;89:258-66.
28. Hunt PA, Jacobs PA. In vitro growth and chromosome constitution of placental cells. II. Hydatidiform moles. Cytogenet Cell Genet 1985;39:7-13.
28a. Vejerslev LO, Dissing J, Hansen HE, Poulsen H. Hydatidiform mole: genetic markers in diploid abortuses with macroscopic villous enlargement. Cancer Genet Cytogenet 1987 (in press).
29. Vejerslev LO, Tommerup N. Hallberg A. Hydatidiform moles: Methods for culture and cytogenetic analyses. Cancer Genet Cytogenet 1986;22:19-27.
30. Honda J, Ikara S, Sarto E, Namoto K, Fukushima T. Hydatidiform mole concurrent with normal pregnancy (twins) following clomiphene therapy. Acta Obstet Gynaecol Jpn 1980;32:2041-3.
31. Fisher RA, Sheppard DM, Lawler SD. Twin pregnancy with complete hydatidiform mole (46,XX) and fetus (46,XY): genetic origin proved by analysis of chromosome polymorphisms. Br Med J 1982;284:1218-20.
32. Ohama K, Ueda K, Okamoto E, Fujiwara A. Two cases of dizygotic twins with androgenetic mole and normal conceptus. Hiroshima J Med Sci 1985;34:371-5.
33. Vejerslev LO, Dueholm M, Hassing Nielsen F. Hydatidiform mole: Cytogenetic marker analysis in twin gestation. Report of two cases. AM J OBSTET GYNECOL 1986;155:614-7.
34. Berkowitz RS, Sandstrom M, Goldstein DP, Driscoll SG. 45,X complete hydatidiform mole. Gynecol Oncol 1982;14:279-83.
35. Womack C, Elston CW. Hydatidiform mole in Nottingham: a 12-year retrospective epidemiological and morphological study. Placenta 1985;6:93-106.

BASIC SCIENCE SECTION

Magnetic resonance imaging of monkey placenta with manganese enhancement

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Magnetic resonance imaging of pregnant cynomolgus monkeys in the last trimester was done on a Picker instrument (Picker International, Inc., Northford, Connecticut) operating at 0.26 tesla. Both inversion-recovery and spin-echo proton images were obtained, demonstrating good detail of fetal and maternal anatomy. The placenta was particularly easy to identify, and its image was further enhanced by a bolus infusion of manganese chloride. Manganese is one of several paramagnetic ions that shortens proton relaxation times, and as a result alters signal intensity in magnetic resonance imaging. An infusion of 10 μmol/kg of manganese chloride was chosen on the basis of an in vitro experiment demonstrating shortening of the spin-lattice relaxation time at increasing concentrations of manganese. Additional studies showed rapid serum clearance of manganese after an intravenous dose with subsequent rapid uptake in the placenta. These results demonstrate that manganese is potentially suitable for studying placental blood flow with magnetic resonance imaging. (AM J OBSTET GYNECOL 1987;157:185-9.)

Key words: Magnetic resonance imaging, pregnancy, nonhuman primates, placenta, manganese chloride enhancement

Magnetic resonance imaging (MRI) is a noninvasive imaging modality that is quickly becoming a popular diagnostic tool. The advantage of MRI over other traditional imaging techniques is that image acquisition does not require ionizing radiation, and differences in soft tissue are clearly delineated. The basic principle of MRI involves alignment of hydrogen nuclei within the subject by an external magnetic field followed by perturbation of this alignment by radio frequency waves. The various radio frequency waves are absorbed and transferred by the hydrogen nuclei to the molecular lattice or other nuclear magnetic moments in the tissue. Overall, macroscopic magnetic moments are then measured and computer processed to form an image partially on the basis of (1) the concentration of hydrogen nuclei; (2) the spin-lattice and the spin-spin relaxation times in different environments within the subject, that is, in fat versus muscle versus bone; and (3) the time variations between radio frequency perturbation and signal acquisition.[1] The radio frequency used to probe nuclei in the subject or tissue of interest can be modified by the strength of the external magnetic field and by the nuclear spin chosen (for example, ^{1}H, ^{13}C, ^{31}P). In addition to imaging, MRI principles can be used as a noninvasive method to study blood flow.[2]

Because of its noninvasive and nonionizing qualities, MRI may be ideal for obstetric imaging. Although there are no known significant risks to the fetus, its use in obstetrics has been confined mostly to the study of abnormal fetuses.[3-7] We have chosen to study and establish the use of this technique in nonhuman primate pregnancies with special emphasis on the placenta because of the eventual possibility of studying blood flow and placental function.

It is known that the proton relaxation times (T_1 and T_2) can be shortened in vivo by paramagnetic ions. Such ions include manganese, gadolinium, iron, copper, nickel, and cobalt.[8] These ions shorten the relaxation rates of the hydrogen nuclei by increasing the efficiency of energy transfer from nuclear spins to the surrounding molecular lattice and from nuclear spin to nuclear spin. These relaxation rates are characterized by spin-lattice and spin-spin measurements, which reflect the time taken for the nuclear population to return to equilibrium or to the unperturbed state, through interactions with the environment (spin-lattice) and neighboring nuclei (spin-spin). As a way of enhancing signal intensity and thus contrast, we also studied the effects of manganese on the placenta. Manganese was chosen because it is a trace metal required for various enzymatic activity.[9]

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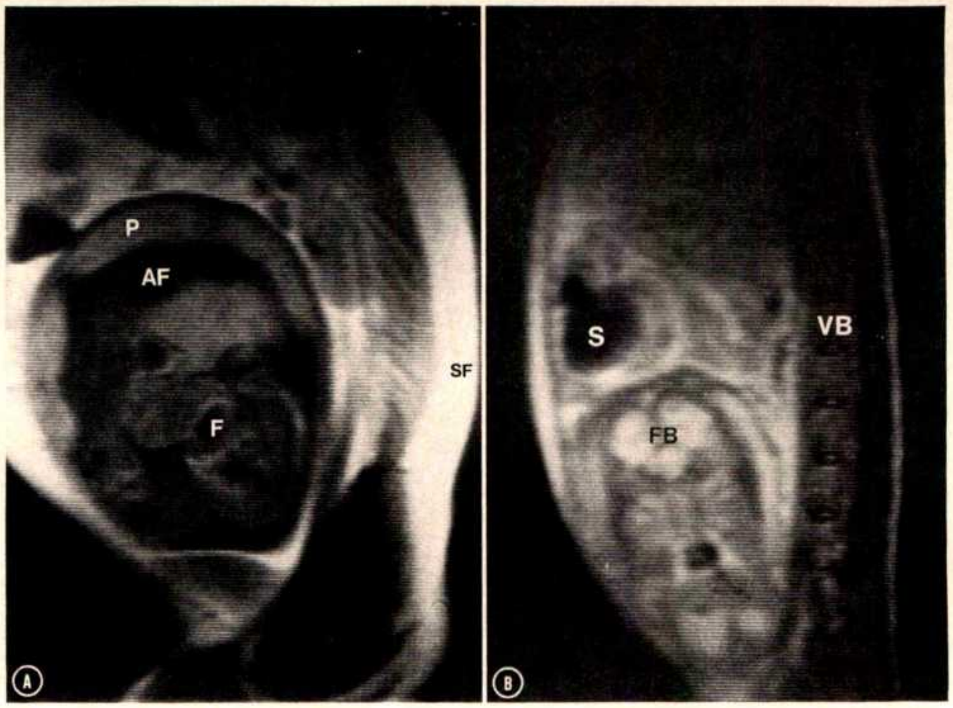

Fig. 1. Spin-echo sagittal images of pregnant cynomolgus monkey with a repetition time/time to echo of 900/40 and 1400/40 msec. *A*, Note that both placental lobes can be visualized with a thin portion of amniotic fluid between. The fetal facial features can be easily discerned on this image. *B*, Both cerebral hemispheres and cerebellum are seen. *P* = Placenta, *F* = fetus, *AF* = amniotic fluid, *FB* = fetal brain, *S* = maternal stomach, *VB* = vertebral body, *SF* = subcutaneous fat.

Material and methods

A Picker magnetic resonance imager (Picker International, Inc., Northford, Connecticut) with a superconducting magnet operating at 0.26 tesla was used in these experiments. Both inversion-recovery and spin-echo scans were obtained. A spin-echo pulse sequence with a repetition time of 600 msec and a time to echo of 40 msec was chosen for most of the scans. The images were obtained with the animals placed in the head coil.

Three cynomolgus monkeys in the last trimester of pregnancy were imaged with MRI. They were anesthetized with ketamine (10 mg/kg intramuscularly) and pentobarbital (10 to 20 mg/kg intravenously). A butterfly needle was inserted for intravenous access, and this was connected to a slow saline solution infusion. The monkeys were positioned on their side for imaging.

Manganese uptake and clearance from serum were studied in two nonpregnant cynomolgus monkeys and one pregnant monkey. After intravenous access and arterial access were established, the monkeys were given an intravenous injection of 100 μCi of manganese-54, a γ-emitting isotope. The isotope was in a 0.5 mol/L hydrochloric acid solution that was diluted with buffered saline solution. At various intervals spanning 60 minutes, blood samples were withdrawn, centrifuged, and 0.1 ml of serum was counted in a γ-scintillation counter. Results were expressed as counts per minute. Immediately after the last sampling at 60 minutes, the animals were killed with an overdose of barbiturate. Tissues were removed from the pregnant monkey, weighed and counted in the γ-counter to determine manganese uptake, and expressed as counts per minute per gram of tissue.

To determine the optimal serum concentration of manganese for image enhancement, an in vitro experiment was conducted. Solutions of manganese chloride in water were made ranging in concentration from 1.4×10^{-9} to 1.4×10^{-3} mol/L. These were taken as theoretic serum concentrations of manganese in animals given manganese chloride ranging from 1×10^{-9} to 1×10^{-2} mol/kg. We assumed each animal weighed approximately 2 kg, and approximately 70% of the weight was body water. Example:

$$\frac{1 \times 10^{-9} \text{ mol/kg} \times 2 \text{ kg}}{2 \text{ kg} \times 0.70 \text{ L/kg}} = \frac{1}{0.70} \times 10^{-9} \text{ mol/L} = 1.4 \times 10^{-9} \text{ mol/L}$$

Spin-lattice values for each solution were determined in a laboratory tabletop nuclear magnetic resonance instrument (PS-100, IBM Instruments Inc., Danbury, Connecticut), which did not perform spin-spin measurements. On the basis of these results, a solution of

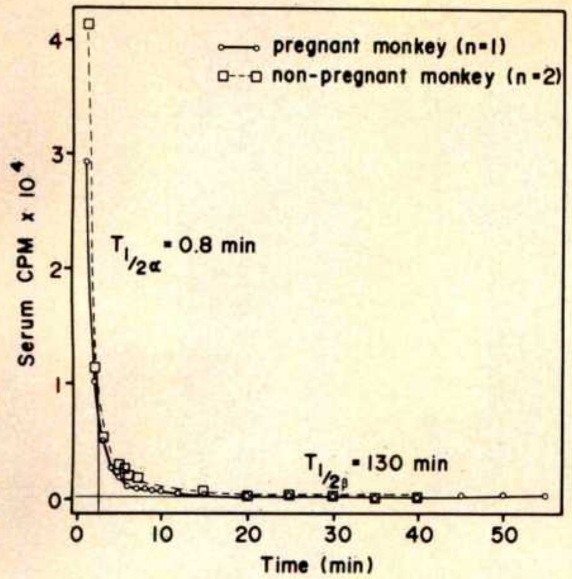

Fig. 2. Serum manganese-54 clearance curves from two nonpregnant monkeys (mean) and one pregnant monkey. The biexponential equation describing both of these sets of data is:

$$A(t) = 233.44 \exp(-0.759t) + 572.85 \exp(-0.576t)$$

derived at with a least-squares curve-fitting program on the mathematical modeling laboratory (MLAB) software for the DEC 10 computer system at National Institutes of Health.

manganese chloride, 10 μmol/kg, would yield a theoretic serum concentration of 1.4×10^{-5} mol/L, the almost minimal concentration required to shorten the spin-lattice measurement. This concentration was chosen for our image enhancement. We anticipated that this serum concentration would enhance placental imaging because the placenta is vascular and rich in mitochondria.

Manganese enhancement of placental images was tested by infusing 10 μmol/kg of manganese chloride as a single intravenous bolus into an anesthetized pregnant monkey. Images were taken serially over several minutes after injection. The animal was then allowed to awaken and was returned to its cage.

Results

With inversion-recovery and spin-echo pulse sequences, images were obtained of the pregnant monkey. Coronal, sagittal, and transverse images were obtained without repositioning. Fig. 1, *A* and *B*, demonstrates spin-echo images of a pregnant monkey in a sagittal view. The intrauterine contents were easily identified, including the placenta, the fetus, and the amniotic fluid. Maternal structures were also identified, including the stomach, marrow in the vertebral bodies, and subcutaneous fat, which gave the brightest signals because of its high proton content and short spin-lattice and spin-spin relaxation times. Muscle and bone gave lower intensity signals because of their lower proton content and longer spin-lattice and spin-spin measurements. With longer repetition times (Fig. 1, *B*), the fetal brain, which has an abundance of lipid, becomes bright, as does the subcutaneous fat. Typically, the cynomolgus monkeys have bilobular placentas that were easily identified in magnetic resonance images.

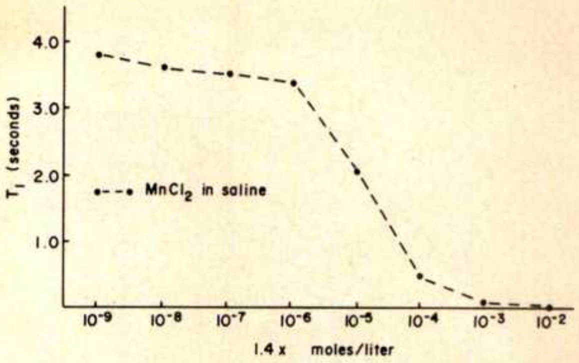

Fig. 3. Spin-lattice measurements of manganese chloride solutions with increasing concentrations.

Table I. Manganese-54 uptake in pregnant cynomolgus monkeys

Tissue	*Pregnant (cpm/gm)*	*Nonpregnant (cpm/gm)*
Liver	359,841	383,135
Spleen	121,127	122,343
Right kidney	212,676	402,604
Left kidney	245,782	303,517
Heart	88,220	67,276
Muscle	3,172	3,281
Uterus	14,160	18,902
Placenta (central)	117,201	
Placenta (marginal)	32,815	
Placenta (marginal)	6,451	

Animals were treated with 100 μCi manganese-54 in 0.5 mol/L hydrochloric acid solution containing 4×10^7 cpm and killed at 60 minutes after treatment. These are the same animals used for the pharmacokinetic studies illustrated in Fig. 2.

Serum clearance studies of manganese in the pregnant and nonpregnant monkeys were almost identical and very rapid. A biphasic clearance pattern is shown (Fig. 2). The first phase had a half-life of 0.8 minute and represented distribution and rapid clearance from the circulation; the second phase had a half-life of approximately 130 minutes and represented total body excretion of the ion. The rapid serum clearance is a result of rapid tissue uptake and renal excretion. The liver, spleen, kidneys, and placenta have the highest concentration of manganese (Table I). The central portion of the placenta, in the region of umbilical cord insertion, had higher manganese concentrations than peripheral regions.

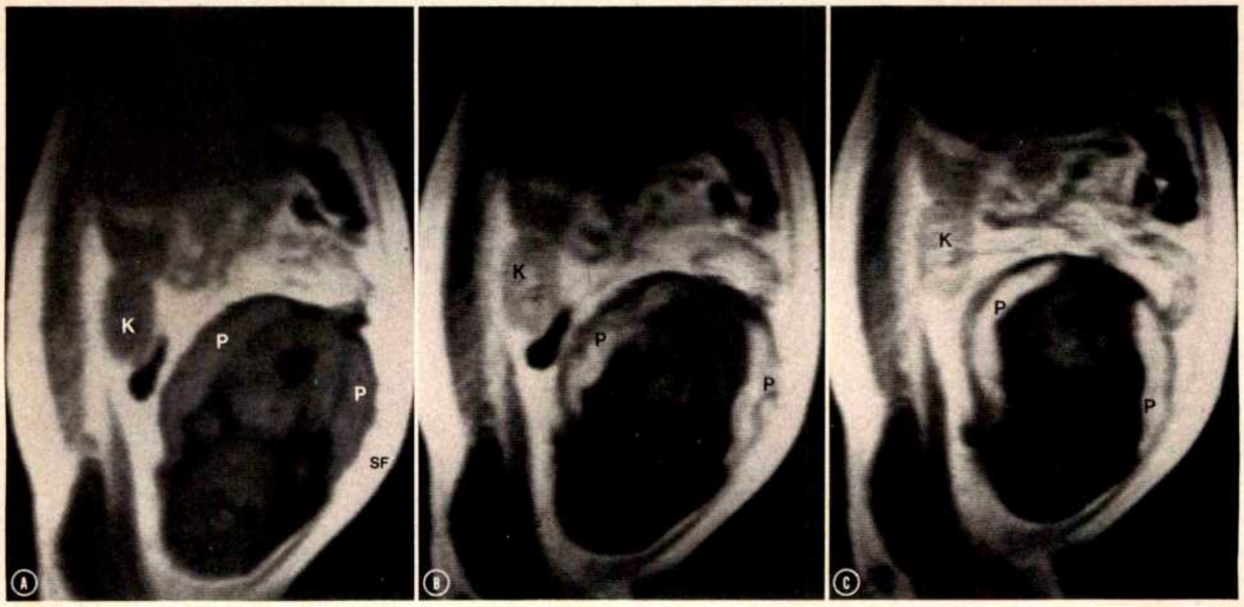

Fig. 4. Spin-echo images of pregnant cynomolgus monkey before and after injection of 10 μmol/kg manganese chloride. *A*, Before injection. *B*, Three minutes after injection. *C*, Five minutes after injection. *P* = Placenta, *K* = maternal kidney, *SF* = subcutaneous fat.

Fig. 3 demonstrates that the shortening of the spin-lattice values begins in solutions that have a concentration of approximately 1.4×10^{-6} mol/L. A serum concentration of 1.4×10^{-5} mol/L was aimed for in our imaging studies. This translated to an approximate dose of 10 μmol/kg, which assumes that the concentration of manganese in the placenta itself is close to or higher than that in serum. The results after infusion of manganese are demonstrated in Fig. 4, *A*, *B*, and *C*, after the monkey was injected with 10 μmol/kg of manganese chloride. Within 5 minutes increased intensity was detected from the placenta compared with the surrounding tissues. The kidney image was also enhanced, suggesting renal uptake of manganese. The increased placental intensity remained for at least 1 hour and only then showed slight decrease. Follow-up scans at 12, 24, and 48 hours showed persistence of the increased intensity of the placental image. A scan done 1 week after the injection showed no remaining enhancement. The architecture within the placenta was somewhat better defined with this ion than in nonenhanced images. The internal regions of the placenta appeared to become enhanced before the rest of the placenta.

Comment

The easily identifiable intrauterine contents and the clearly defined maternal anatomy on MRI in our studies are comparable to the results reported by other investigators studying human pregnancies.[3-7] This detail is superior to the image generated by ultrasound examination because of low volumes of amniotic fluid, which make ultrasound evaluation difficult. Because the teratogenic effects, if any, of MRI are unknown, the pregnant nonhuman primate may serve as a good model for, and prelude to, human pregnancy studies.

Our results demonstrate the paramagnetic effects of manganese in vivo, particularly in the liver, spleen, kidney, heart, and placenta. Magnetic resonance images of these tissues would be enhanced by an infusion of manganese. With additional studies this ion may prove to be clinically useful in defining the anatomy of certain organs (that is, the placenta), and the presence of abnormal space-occupying lesions such as infarcts may be more easily detected.

Interest has centered around the use of manganese as an agent for myocardial imaging, since ischemic myocardium has lower uptake of manganese.[10, 11] Manganese also appears to be a good ion for studying organ blood flow, particularly for the placenta, because it is so rapidly cleared from the serum. The half-lives from these experiments in nonhuman primates compare well with the reported half-lives of manganese in dogs[11] and humans.[12]

However, the use of manganese as an agent for MRI may be limited by its local toxicity, since it tends to remain in tissue for a long period of time. Repeated dosing for multiple imaging studies may further increase the cumulative effects of the manganese. Manganese toxicity with neurological and psychotic sequelae have been reported among miners chronically exposed to high environmental levels.[13] Taylor and

Price[14] reported a case of nausea and vomiting followed by acute pancreatitis in a patient on dialysis who was exposed to a manganese-contaminated dialysate. Mortality has been reported in guinea pigs, but our current dose of 10 μmol/kg is well below the doses used by other investigators (100 to 200 μmol/kg).[8, 15] Chelating the ion with ethylenediaminetetraacetic acid, as suggested by Mendonca-Dias et al.,[8] may decrease its toxicity by increasing its renal clearance, and this should be explored further. Another suggested alternative would involve giving manganese orally rather than parenterally.[16]

Regional differences were seen in the MRI of these cynomolgus placentas with the central regions appearing to be enhanced before the rest of the organ. However, this was not a striking finding possibly because the placenta is vascular and manganese is rapidly distributed throughout the organ. Further studies are needed, but this paramagnetic ion may prove to be useful for studying regional blood flow in the placenta.

In conclusion, we have been able to demonstrate that MRI of the pregnant nonhuman primate is feasible with good visualization of maternal and fetal anatomy. For our interests the placenta was easily identified. We have also been able to demonstrate that manganese, a paramagnetic ion, can enhance the image of the placenta and other organs. An intravenous dose of manganese chloride (10 μmol/kg) gave prompt enhancement of the placental image. The rapid flow of blood through the intervillous space of the placenta, however, makes it appear homogeneous, and its internal architecture becomes more difficult to study. Because of its rapid serum clearance, manganese may be used to study placental blood flow, especially since uptake is rapid in that organ. Recent experiments in vitro have demonstrated human placental uptake of manganese with a subsequent shortening of the spin-lattice measurement consistent with the observed brightening of the placental image in cynomolgus monkeys (Plowchalk DR, Jordan JP, Thomford PJ, and Mattison DR, unpublished observations). This suggests that manganese may be useful in human placental MRI. However, the use of manganese may be limited by its persistence in the placenta and its potential for toxic side effects, especially central nervous system toxicity.

REFERENCES

1. Pykett IL. NMR imaging in medicine. Sci Am 1982; 246:78-88.
2. Singer JR, Crooks LE. Nuclear magnetic resonance blood flow measurements in the human brain. Science 1983; 221:654-6.
3. Johnson IR, Symonds EM, Kean DM, et al. Imaging the pregnant human uterus with nuclear magnetic resonance. AM J OBSTET GYNECOL 1984;148:1136-9.
4. Lowe TW, Weinreb J, Santos-Ramos R, Cunningham FG. Magnetic resonance imaging in human pregnancy. Obstet Gynecol 1985;66:629-33.
5. McCarthy SM, Filly RA, Stark DD, et al. Obstetrical magnetic resonance imaging. Fetal anatomy. Radiology 1985;154:427-32.
6. Smith FW, MacLennan F, Abramovich DR, MacGilivray I, Hutchinson JMS. NMR imaging in human pregnancy: a preliminary study. Magn Reson Imaging 1984; 2:57-64.
7. Smith FW, Kent C, Abramovich DR, Sutherland HW. Nuclear magnetic resonance imaging—a new look at the fetus. Br J Obstet Gynaecol 1985;92:1024-33.
8. Mendonca-Dias MH, Gaggelli E, Lauterbur PC. Paramagnetic contrast agents in nuclear magnetic resonance medical imaging. Semin Nucl Med 1983;13:364-76.
9. Underwood EJ. Trace elements in human and animal nutrition. 4th ed. New York: Academic Press, 1977: 170-95.
10. Chauncey DM, Schelbert HR, Halperin SE, et al. Tissue distribution studies with radioactive manganese: a potential agent for myocardial imaging. J Nucl Med 1977; 18:933-6.
11. Atkins HL, Som P, Fairchild RG, et al. Myocardial positron tomography with manganese −52 m. Radiology 1979; 133:769-74.
12. Borg DC, Cotzias GC. Manganese metabolism in man: rapid exchange of Mn^{56} with tissue as demonstrated by blood clearance and liver uptake. J Clin Invest 1958; 37:1269-78.
13. Cotzias GC, Horiuchi K, Fuenzalida S, Mena I. Chronic manganese poisoning. Neurology 1968;18:376-82.
14. Taylor PA, Price JD. Acute manganese intoxication and pancreatitis in a patient treated with a contaminated dialysate. Can Med Assoc J 1982;126:503-5.
15. Wolf FL, Baum L. Cardiovascular toxicity and tissue proton T_1 response to manganese injection in the dog and rabbit. AJR 1983;141:193-7.
16. Burnett KR, Goldstein EJ, Wolf GL, Sen S, Mamourian AC. The oral administration of $MnCl_2$: a potential alternative to IV injection for tissue contrast enhancement in magnetic resonance imaging. Magn Reson Imaging 1984; 2:307-14.

Hypoglycemia: How little is too much for the embryo?

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The effects of hypoglycemia on mammalian embryos undergoing neurulation (third to fourth week of human development) were investigated. Mouse embryos were maintained for 28 hours in whole embryo culture in serum collected from rats that had received 50 units of 100 United States Pharmacopeia insulin units per milliliter. Glucose concentrations used were 40, 60, 80, and 147 mg/dl (normal blood glucose in the pregnant mouse is 125 mg/dl). After the culture period embryos were evaluated for malformations and growth and compared with those maintained under euglycemic conditions. The results demonstrate that glucose concentrations approximately 50% of normal maternal levels were teratogenic but not growth inhibitory, whereas concentrations in the range of 30% to 40% of maternal levels were lethal to the embryo. Furthermore, a 14-hour exposure to reduced blood sugar in either the first or second half of the culture period produced malformations. (AM J OBSTET GYNECOL 1987;157:190-3.)

Key words: Hypoglycemia, congenital malformations, embryo, neurulation

In recent years there has been a proliferation of investigations into the factors responsible for the increased incidence of congenital malformations among infants of diabetic mothers. These studies[1-3] have focused on factors characteristically altered in the poorly controlled insulin-deficient diabetic patient, such as hyperglycemia, ketoacidosis, hypoinsulinemia, and have documented that the severity of the disease and the adequacy of insulin therapy to control the diabetic state play a role in the incidence of congenital defects. Clinical trials[4] have also documented the link between good control and a reduced incidence of malformations, and treatment protocols now mandate that strict control of the disease instituted before conception and maintained throughout pregnancy will reduce the risk to the offspring. Although clinical evidence[5] strongly supports such an approach, overaggressive attempts to prevent hyperglycemia may in fact result in a higher frequency of hypoglycemic episodes, as occurs for example with the use of insulin pumps.[6] The occurrence of such episodes at stages of embryogenesis that are sensitive to reduced glucose concentrations could prove as disastrous to the well-being of the unborn offspring as those factors that are altered when insufficient insulin is provided. Therefore the teratogenic effects of hypoglycemia during the period of neurulation in mouse embryos were investigated in the system of whole mammalian embryo culture.

Material and methods

Randomly bred ICR mice were maintained on a 12-hour-light/12-hour-dark cycle (7:00 AM to 7:00 PM), provided with laboratory food and water ad libitum, and mated overnight. The next morning presence of a vaginal sperm plug was considered evidence of mating and was designated day 1 of pregnancy. On day 9, pregnant mice were killed, uterine horns were removed, and embryos were prepared for whole embryo culture in the manner described previously.[7] Embryos were freed from the uterine horns, and the trophoblast, parietal yolk sac, and Reichert's membrane were removed with watchmaker's forceps. Only embryos of three to five somite pairs were placed into 30 ml culture flasks containing 3 ml of medium, gassed with 5% oxygen, 5% carbon dioxide, and 90% nitrogen, and placed on a rotator wheel (30 rpm) at 37° C.

Culture medium consisted of 75% immediately centrifuged heat-inactivated rat serum[8] and 25% Tyrode's solution. Serum was collected from retired breeder Sprague/Dawley rats by aortic puncture; anesthetic ether was administered. Normoglycemic serum was collected from untreated rats and had a final glucose concentration of 147 mg/dl. Hypoglycemic serum was obtained by injecting rats intraperitoneally with 50 units of 100 United States Pharmacopeia insulin units per milliliter and collecting the serum 3 to 4 hours later. Final glucose concentrations from hypoglycemic rats were 34 mg/dl. Glucose concentrations in medium used for culture were adjusted by diluting serum with Tyrode's solution, which contained no glucose, and by supplementation of the medium with additions of a β-D-glucose solution in distilled water (50 mg/ml). Glucose concentrations were monitored at 0 hours and 28 hours with a glucose 2 analyzer (Beckman Instruments Inc., Fullerton, California).

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Table I. Results of experiments in rat embryos

Glucose concentration (mg/dl)		*Malformations*			
Initial	*Final*	*%*	*N*	*Somites (±SE)*	*Protein (μg ± SE)*
147	109	0	9/10	18.2 ± 0.2	105.3 ± 6.2
80	54	22	2/9	20.3 ± 0.4	99.4 ± 6.2
60	38	90*	9/10	19.8 ± 0.3	89.6 ± 5.9
40	28	100*	8/8	—	37.6 ± 6.6*
80—60		78*	8/9	19.2 ± 0.6	99.6 ± 7.3
60—80		50*	4/8	19.2 ± 0.9	119.8 ± 10.8

*$p < 0.05$.

With the protocols described above, three to five somite embryos were cultured for 28 hours in medium prepared from normoglycemic and hypoglycemic animals containing different glucose concentrations (Table I), that is, 147 mg/dl (normoglycemic) and 40, 60, and 80 mg/dl (hypoglycemic). In addition, one group of embryos was cultured in serum collected from hypoglycemic rats but with glucose concentrations adjusted to normoglycemic levels (147 mg/dl). Finally, two groups of embryos were transferred after 12 hours from medium at one glucose concentration to medium at another, that is, one group started in medium at 60 mg/dl and was then transferred to medium containing 80 mg/dl, and another group was started in medium at 80 mg/dl and then was transferred to medium containing 60 mg/dl glucose. After 28 hours of culture, all embryos were examined under a dissecting microscope for malformations, heartbeat and circulation, and total somites. Embryos with no heartbeat or circulation were termed *failed to thrive*. Total embryonic protein was also determined with the Lowry method. Statistical analyses were performed with an analysis of variance.

Results

Embryos maintained in serum collected from normoglycemic rats progressed normally from the three to five to 18 to 19 somite stage of development in 28 hours of culture (Fig. 1). During this time a heartbeat and circulation were established and the initially open neural tube was completely closed from the cranial region down to the last somite pair, where the posterior neuropore remained open but appeared normal. Total embryonic protein increased to 105 ± 6 μg/embryo (Table I). Overall, this growth and development paralleled that observed in embryos in vivo over the same time period as has been reported previously.[7]

The morphology and growth of embryos maintained in serum collected from hypoglycemic rats were dependent on the concentration of glucose present (Table I). Thus 75% of embryos cultured in medium initially containing 40 mg/dl glucose failed to thrive, and all were severely malformed and growth retarded. Embryos grown in medium initially containing 60 mg/dl showed a high incidence of neural tube and facial defects, although total embryonic protein was not significantly less than that observed in embryos maintained in normoglycemic medium. Malformations in the 60 mg/dl group included a lack of closure of the cranial neuropore in the forebrain and midbrain regions and hypoplasia of the forebrain and maxillary division of the first visceral arch (Fig. 2). Embryos maintained in medium initially containing 80 mg/dl glucose and those grown in serum from hypoglycemic animals supplemented with glucose to concentrations equal to normoglycemic serum (147 mg/dl) showed no significant changes in malformation rates, somite numbers, or total embryonic protein compared with embryos cultured in medium prepared from non–insulin-treated rats. However, two embryos (22%) cultured in medium containing 80 mg/dl glucose suffered facial defects similar to those observed in the 60 mg/dl group, and one of these also had an open cranial neuropore.

In experiments in which embryos were transferred after 12 hours either from cultures containing 80 mg/dl glucose to those with 60 mg/dl or from those with 60 to those with 80 mg/dl, malformation rates were increased compared with rates in embryos cultured in serum from normoglycemic rats or hypoglycemic serum supplemented with glucose to control concentrations (147 mg/dl) (Table I). The malformations were identical to those observed in the 60 mg/dl group and involved the cranial region. No decrease in somite numbers or total embryonic protein was observed in embryos involved in these transfer experiments.

Comment

The period of neurulation in the mouse extends from the time of early elevation of the neural folds (day 8.5; plug day is day 1) to complete closure of the neural tube at 25 somites (day 10.5). This stage of development is equivalent to days 19 to 28 (after fertilization) in the human and represents an extremely sensitive time in development when teratogenic insults frequently occur, especially among diabetic women.[9] On the basis of re-

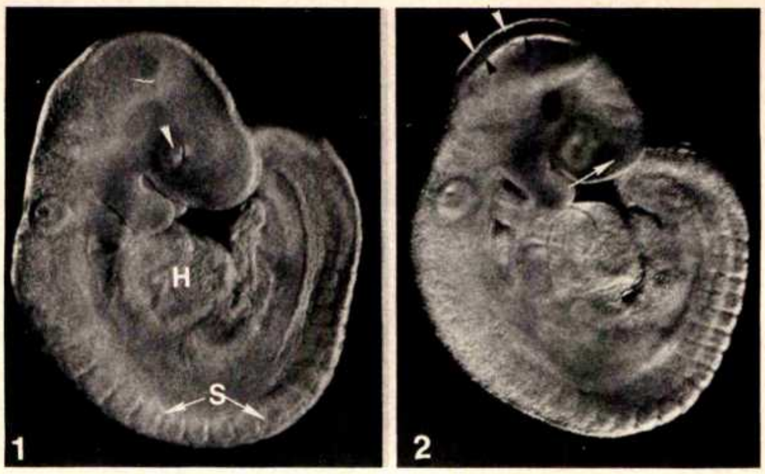

Fig. 1. Embryo cultured 28 hours in normoglycemic (147 mg/dl glucose) medium showing 19 to 20 somite pairs and well-defined brain vesicles and visceral arches. Heart *(H)*, somites *(S)*, optic vesicle *(arrowhead)*. (× 132.)

Fig. 2. Embryo cultured 28 hours in hypoglycemic (60 mg/dl glucose) medium showing 19 to 20 somite pairs and similar visceral arch development to the specimen in Fig. 1. However, the cranial neural folds have failed to close *(arrowheads)*, and the forebrain region and maxillary process are hypoplastic *(arrows)*. (× 132.)

sults from the present study, it appears that hypoglycemia is teratogenic to neurulating embryos and can produce growth retardation if the concentration of glucose is sufficiently reduced. However, the results also demonstrate that growth retardation, at least for the period of time tested in this study, is not a prerequisite for the production of hypoglycemia-induced embryopathies. The observation that the addition of sugar to serum from insulin-treated severely hypoglycemic animals (40 mg/dl glucose) restored normal embryonic growth and development demonstrates that the lack of glucose is the teratogenic insult. Thus other serum factors that might change after administration of insulin, such as glucagon, are not producing the malformations observed in this study.

The fact that rodent embryos require glucose in whole embryo culture has been reported previously by investigators using human serum[10, 11] and serum from unfed rats[12] as culture medium. Initial glucose concentrations in these studies varied from 60 to 80 mg/dl, which led to malformed and growth-retarded embryos. With the addition of exogenous glucose to concentrations of 120 to 300 mg/dl, normal growth was restored. The fact that moderate hyperglycemia is used in embryo culture is the result of two phenomena. (1) The culture technique uses a closed system with a finite volume of culture medium, that is, 1 to 5 ml. Therefore, as the embryo uses glucose from the medium, the sugar is not replaced, and consequently the concentration decreases (Table I), thereby necessitating the initiation of cultures with an adequate supply of sugar to last the entire culture period. (2) In most laboratories serum is collected from rats by using anesthetic ether, which produces stress. In turn, this stress elicits a release of epinephrine, which produces a glycogenolytic cascade that results in elevated serum glucose concentrations. Fortunately, moderate hyperglycemia is not teratogenic to rodent embryos in culture.[13, 14]

From a biochemical standpoint glucose appears to be an essential energy source during the earliest stages of embryogenesis, that is, gastrulation to early limb bud stages.[15] During the period of neurulation greater than 90% of all glucose used passes through the glycolytic pathway and, unlike the situation in adult and fetal tissues, is converted aerobically to lactate.[16, 17] Another 2% of the total glucose consumed is metabolized to ribose via the pentose phosphate pathway to be used in the de novo synthesis of nucleosides. Finally, only 0.2% of the glucose used passes through the Krebs' cycle, which has a low activity during neurulation.[16, 17] Thus glucose plays an important role as an energy source and anabolic substrate at these early developmental stages, and it is not surprising that insufficient supplies of this sugar place the embryo at risk of developing abnormally.

It should be noted that all of the work cited has been obtained from studies with rodent embryos and not humans. In this regard, species differences exist in the responses of mouse versus rat embryos to conditions of altered glucose concentrations.[13, 14] Despite these differences, however, the potential for hypoglycemia to disrupt normal mammalian embryogenesis appears likely if glucose concentrations are reduced sufficiently during critical phases of development when the sugar is an essential metabolic substrate. The degree of hypoglycemia that can be tolerated by the embryo will also depend on the duration of exposure to reduced blood sugar concentrations. As a point of reference, in the mouse normal maternal blood glucose concentration is approximately 125 mg/dl and malformations were observed in embryos maintained in the range of 40 to 80 mg/dl (Table I). Thus glucose concentrations ranging from 30% to 50% of normal maternal blood sugar levels for as little as a 12-hour exposure are detrimental to the rodent embryo during neurulation. Therefore, on the basis of this murine model, human embryonic development may be at risk if severe episodes of maternal hypoglycemia occur during the third to fourth weeks of gestation.

REFERENCES

1. Horii K, Watanabe G, Ingalls TH. Experimental diabetes in pregnant mice. Prevention of congenital malformations in offspring by insulin. Diabetes 1966;15:194.
2. Eriksson U, Dahlstrom E, Larsson KS, Hellerstrom C. Increased incidence of congenital malformations in the offspring of diabetic rats and their prevention by maternal insulin therapy. Diabetes 1982;31:1.
3. Sadler TW, Horton WE Jr. Effects of maternal diabetes on early embryogenesis: the role of insulin and insulin therapy. Diabetes 1983;32:1070.
4. Fuhrman K, Reiher H, Semmler K, Fischer F, Fischer M, Glockner E. Prevention of congenital malformations in infants of insulin-dependent diabetic mothers. Diabetes Care 1983;6:219.
5. Freinkel N, Dooley SL, Metzger BE. Care of the pregnant woman with insulin-dependent diabetes mellitus. N Engl J Med 1985;313:96.
6. Gabbe SG. Management of diabetes mellitus in pregnancy. AM J OBSTET GYNECOL 1985;153:824.
7. Sadler TW. Culture of early somite mouse embryos during organogenesis. J Embryol Exp Morphol 1979;49:17.
8. Steele CE, New DAT. Serum variants causing the formation of double hearts and other abnormalities in explanted rat embryos. J Embryol Exp Morphol 1974; 31:707.
9. Mills JL, Baker L, Goldman AS. Malformations in infants of diabetic mothers occur before the seventh gestational week: implications for treatment. Diabetes 1979;28:292.
10. Chatot CL, Klein NW, Piatek J, Pierro LJ. Successful culture of rat embryos in human serum: use in the detection of teratogens. Science 1980;207:1471.
11. Gupta M, Beck F. Growth of 9.5 day rat embryos in human serum. J Embryol Exp Morphol 1983;76:1.
12. Ellington SKL. In-vivo and in-vitro studies on the effects of maternal fasting during embryonic organogenesis in the rat. J Reprod Fertil 1980;60:383.
13. Cockroft DL, Coppola PT. Teratogenic effects of excess glucose on headfold rat embryos in culture. Teratology 1977;16:141.
14. Sadler TW. Effects of maternal diabetes on early embryogenesis: II. Hyperglycemia-induced exencephaly. Teratology 1980;21:349.
15. Shepard TH, Tanimura T, Robkin MA. Energy metabolism in early mammalian embryos. Dev Biol 1970;4 (suppl):42.
16. Tanimura T, Shepard TH. Glucose metabolism by rat embryos in vitro. Proc Soc Exp Biol Med 1970;135:51.
17. Neubert D, Peters H, Teske S, Kohler E, Barach HJ. Studies on the problem of aerobic glycolysis occurring in mammalian embryos. Naunyn Schmiedebergs Arch Pharmacol 1971;268:234.

Cyclooxygenase inhibition reduces placental transfer: Reversal by carbacyclin

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The effect of the cyclooxygenase inhibitors, indomethacin and ibuprofen, on diffusional transfer in the human placenta was assessed with the dual-perfused isolated placental lobe. Antipyrine, a freely diffusible substance, was used as an indicator of placental transfer efficiency. Each inhibitor (100 μmol/L) was perfused for 30 minutes after a baseline period, resulting in a significant reduction in antipyrine clearance. During a subsequent washout period, ibuprofen-inhibited antipyrine clearance returned to baseline values, whereas indomethacin-inhibited clearance remained reduced. An additional 30 minutes perfusion of 500 μmol/L of ibuprofen resulted in a further reduction in antipyrine clearance compared with 100 μmol/L of ibuprofen, suggesting a dosage-related effect. The perfusion of each inhibitor caused a reduced production of 6-keto-prostaglandin $F_{1\alpha}$ (the stable metabolite of prostacyclin) in the fetal circulation. The simultaneous perfusion of carbacyclin, a prostacyclin analogue, at 100 nmol/L and 1 μmol/L resulted in a dosage-dependent reversal of the effects of ibuprofen (500 μmol/L) on antipyrine clearance. The results indicate that the inhibition of cyclooxygenase activity reduces placental transfer and that the effects of these inhibitors are reversed by carbacyclin. This study suggests that the use of cyclooxygenase inhibitors during pregnancy could compromise the developing fetus by reducing placental transfer. (AM J OBSTET GYNECOL 1987;157:194-8.)

Key words: Indomethacin, ibuprofen, isolated placental lobe, placental transfer, carbacyclin

The role of prostacyclin in the regulation of human placental blood flow is presently unclear. Two important pathologic conditions in humans, preeclampsia and intrauterine growth retardation (IUGR), are associated with decreased placental perfusion and a concomitant decrease in prostacyclin production.[1] These findings led to the suggestion that a balance between the vasodilator action of prostacyclin and the vasoconstrictor activity of thromboxane A_2 is necessary for the maintenance of normal vascular tone in the pregnant human uterus and placenta.[2] However, two recent clinical findings have cast some doubt on this hypothesis. First, maternal infusion of prostacyclin in normal pregnancies or pregnancies involving preeclampsia apparently does not affect fetoplacental blood flow.[3,4] However, these studies measured only total placental blood flow and could not distinguish possible regional shifts in flow within the tissue. Therefore effects at the maternal-fetal exchange area could not be evaluated. Second, the use of antiplatelet therapy (including cyclooxygenase inhibitors) has been proposed as a therapeutic modality in pregnancies at risk for preeclampsia or IUGR.[5] These studies have suggested that the inhibition of prostaglandin synthesis reverses thrombocytopenia and improves the outcome in these pathologic pregnancies. However, no evaluation of placental transport or blood flow was conducted.

Thus current information suggests that prostacyclin is necessary for the maintenance of umbilical-placental blood flow and that the administration of low dosages of cyclooxygenase inhibitors may improve pregnancy outcome in pathologic states associated with an imbalance in prostacyclin and thromboxane A_2 production. A more fundamental knowledge of the role of prostacyclin as a potential mediator of vascular tone in the small vessels of the terminal placental villi, where maternal-fetal exchange occurs, appears warranted. Moreover, because drug-induced inhibition of cyclooxygenase activity is being advocated in conditions associated with placental insufficiency, the effect of such inhibition on blood flow and placental transport needs to be investigated.

With antipyrine as an indicator of diffusional transfer, we have demonstrated that cyclooxygenase inhibitors significantly reduce placental transfer, and that carbacyclin, a prostacyclin analogue, reverses these effects.

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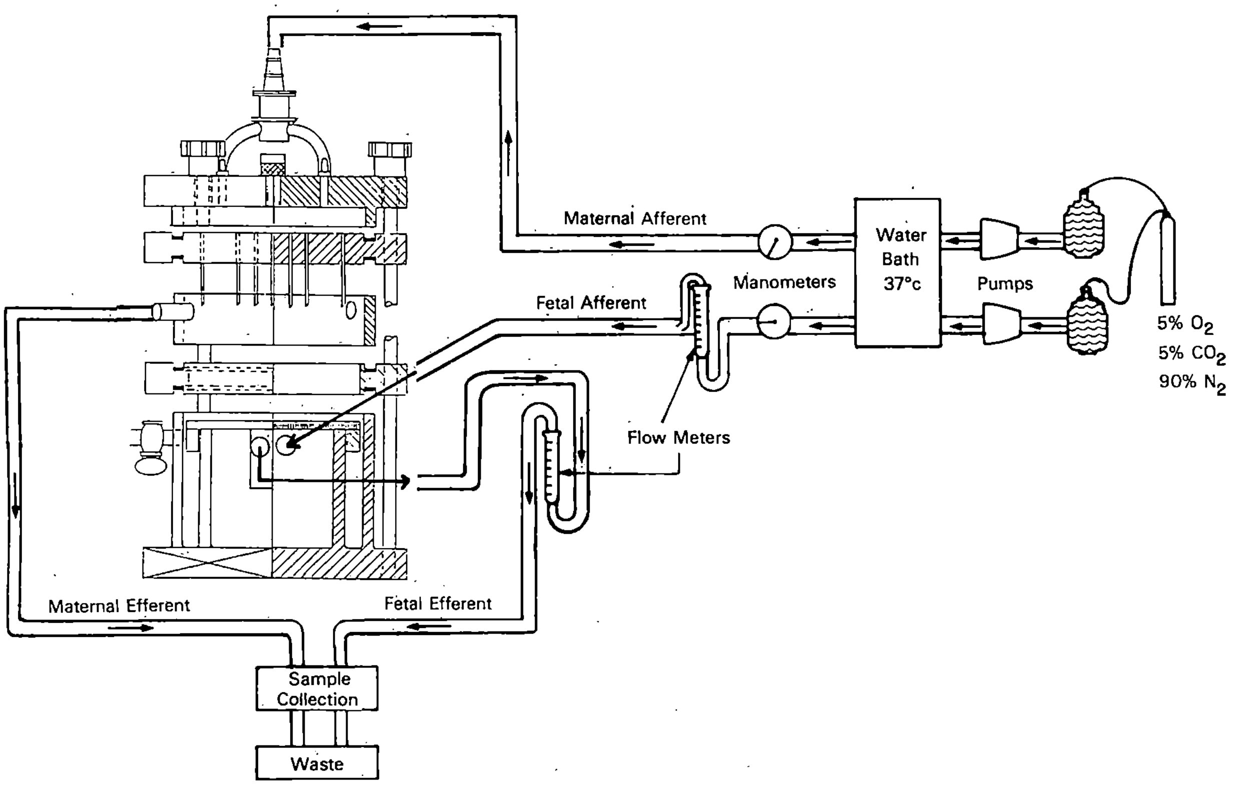

Fig. 1. Perfusion system for an isolated lobe of the human placenta.

Material and methods

Effect of cyclooxygenase inhibitors on placental transfer. Placentas from uncomplicated term pregnancies were obtained with informed consent immediately after vaginal or cesarean section delivery. Placentas from mothers who smoke or from those who ingested cyclooxygenase inhibitors in the 2 weeks before delivery were excluded from the study. A suitable lobe was isolated in a perfusion chamber (Fig. 1), and both fetal and maternal circulations were reestablished within 20 minutes of delivery. Isolated lobes were perfused in a nonrecirculating (open) system with a tissue culture medium (M199, Gibco, Grand Island, New York) containing 30 gm/L dextran (average molecular weight = 80,000) and 1 gm/L glucose.

Perfusate was equilibrated with a gas mixture of 5% oxygen, 5% carbon dioxide, and 90% nitrogen, resulting in afferent arterial oxygen levels of 85 to 90 mm Hg. This gas mixture was chosen to avoid the toxic effects of hyperoxia on the perfused tissue. High oxygen levels have been shown to cause placental vasoconstriction and may also inhibit vascular prostacyclin production.[5] Although placental oxygen consumption in these preparations (1.5 to 1.7 ml/min/kg) is lower than the level reported by other investigators[7] who used buffered perfusates that contained whole blood, it is comparable to systems that use air or 95% oxygen in perfusates without whole blood or red blood cells.[7, 8] Indeed, our preliminary data suggest that hyperoxia (95% oxygen) results in reduced prostacyclin synthesis, increased placental vascular pressure, and reduced antipyrine clearance in the perfused placental lobe.

A fetal flow rate of 7.5 ml/min and a maternal flow rate of 10.2 ml/min resulted in afferent pressures of 25 to 40 and 5 to 15 mm Hg, respectively. Afferent pressures were monitored continuously, and fetal afferent and efferent flow rates were determined to be equal, which confirmed the integrity of the fetal circulation. This preparation has been validated through analysis of tissue histology, hydrogen-3–dextran exclusion, carbon-14–leucine transport, β human chorionic gonadotropin production, and the stability of perfusate pH, oxygen, and carbon dioxide partial pressures.

Each circulation was flushed with 300 ml of perfusate before every experiment. Antipyrine (30 mg/L), a freely diffusible substance, was added to the maternal afferent circulation 15 minutes before each experiment as a marker of placental transfer efficiency. Cyclooxygenase inhibitors (indomethacin and ibuprofen, 100 μmol/L) were perfused through both afferent circulations after a 30-minute baseline period. The concen-

Table I. Effects of cyclooxygenase inhibitors on placental antipyrine clearance

Inhibitor tested	*Baseline*	*Inhibitor (100 μmol/L)*	*Wash*	*Inhibitor (500 μmol/L)*
Indomethacin	1.92	1.45*	1.27†	
(n = 5)	(0.23)	(0.19)	(0.25)	
Ibuprofen	2.28	1.83*	2.30	1.47†
(n = 4)	(0.12)	(0.13)	(0.07)	(0.28)

Data presented as mean antipyrine clearance in milliliters per minute (± SEM). Significance determined by randomized complete blocks in analysis of variance in conjunction with Dunnett's procedure to test differences from baseline values (*$p < 0.05$; †$p < 0.01$).

$$\text{Clearance} = \frac{[\text{Fv}] \times \text{Qf}}{[\text{Ma}]}$$

where [Fv] = antipyrine concentration in fetal vein; Qf = fetal flow rate; [Ma] = antipyrine concentration in maternal artery.

tration of ibuprofen used in this study is comparable to peak serum concentrations (approximately 150 μmol/L) after administration of a single 400 mg tablet. However, because indomethacin is a time-dependent cyclooxygenase inhibitor,[9] we used a concentration higher than would be expected after administration of a 25 mg capsule (approximately 6 μmol/L) to obtain effects in a short period of time. Perfusion of a drug-free medium was conducted for the final 30 minutes (washout period) to determine if the effects on antipyrine clearance and the production of 6-keto-prostaglandin $F_{1\alpha}$ were reversible. In the case of ibuprofen (a reversible cyclooxygenase inhibitor[9]), two dosages were tested so that an additional 30-minute perfusion period with 500 μmol/L of ibuprofen was conducted after the washout period. These latter experiments were conducted to determine if effects on antipyrine clearance were dosage-related.

Samples of fetal venous perfusate were collected at the end of each 30-minute period and subjected to spectrophotometric analysis of antipyrine concentration[10] and specific radioimmunoassay (RIA) of 6-keto-$PGF_{1\alpha}$. Antibody was obtained from Seragen, Inc. (Boston), and IgG sorb was used as the precipitating agent. Samples for 6-keto-$PGF_{1\alpha}$ analysis were partially purified with Sep-pak C-18 reverse-phase cartridges (Waters Chromatography Division, Millipore, Milford, Massachusetts), and 6-keto-$PGF_{1\alpha}$ was eluted with 80% acetonitrile in 0.1% acetic acid. Samples were further purified by reverse-phase high performance liquid chromatography with a Beckman high performance liquid chromatography system (Beckman Instruments, Inc., Fullerton, California) and a C-18 reverse-phase column. Prostaglandins were separated in a mobile phase of 35% acetonitrile in 0.1% acetic acid at a flow rate of 1.1 ml/min. The 6-keto-$PGF_{1\alpha}$ peak was collected and subjected to RIA.

Effect of a prostacyclin analogue on placental transfer. In further experiments, the effect of carbacyclin, a synthetic analogue of prostacyclin, on ibuprofen-inhibited placental transfer was assessed. After a 30-minute baseline period, ibuprofen (500 μmol/L) was added to both afferent circulations. Perfusion was continued for 30 minutes, then carbacyclin (100 nmol/L) was added to both circulations for the subsequent 30 minutes, followed by carbacyclin (1 μmol/L) for the final 30 minutes. These concentrations of carbacyclin are similar to those necessary to elicit effects on adenosine diphosphate–induced platelet aggregation and rat systemic arterial blood pressure.[11] Samples of fetal effluent were collected at the end of each 30-minute period and analyzed for antipyrine concentration.

Data are expressed as mean (±SEM) values and statistical significance compared with baseline values determined by randomized complete blocks in analysis of variance and Dunnett's analysis.

Results

Effect of cyclooxygenase inhibitors on placental transfer. The data presented in Table I demonstrate the effect of indomethacin (100 μmol/L) and ibuprofen (100 and 500 μmol/L) on antipyrine clearance in the perfused placental lobe. Each cyclooxygenase inhibitor reduced the transfer of antipyrine from the maternal to the fetal circulation. Antipyrine clearance remained reduced after the washout of the time-dependent, irreversible[9] (or at least slowly reversible[12]) cyclooxygenase inhibitor, indomethacin. Ibuprofen, a rapidly reversible, competitive inhibitor of cyclooxygenase, produced no persistent effect on antipyrine clearance after the washout period. In addition, perfusion of 500 μmol/L of ibuprofen after the washout period caused a further reduction in antipyrine clearance compared with 100 μmol/L of ibuprofen, suggesting a dosage-related effect. Data from RIA of 6-keto-$PGF_{1\alpha}$ (Fig. 2) showed that perfusion of indomethacin (100 μmol/L) or ibuprofen (500 μmol/L) completely inhibited 6-keto-$PGF_{1\alpha}$ production in the placental vascular bed and that inhibition by ibuprofen was completely reversed after the washout period. However, the production of

Table II. Effect of carbacyclin, a prostacyclin analogue, on ibuprofen-inhibited antipyrine clearance

Baseline	*Ibuprofen (500 µmol/L)*	*+ Carbacyclin (100 nmol/L)*	*+ Carbacyclin (1 µmol/L)*
2.14 (0.16) n = 5	1.62* (0.16)	1.94† (0.14)	2.15 (0.19)

Data presented as mean antipyrine clearance in milliliters per minute (±SEM). Significance determined by randomized complete blocks in analysis of variance in conjunction with Dunnett's procedure to test differences from baseline values (*$p < 0.01$; †$p < 0.05$).

6-keto-PGF$_{1\alpha}$ recovered to only 30% of its baseline value after the washout of the indomethacin.

Effect of a prostacyclin analogue on placental transfer. Table II demonstrates the effects of the prostacyclin analogue, carbacyclin, on ibuprofen-inhibited antipyrine clearance. Inhibition of antipyrine clearance by ibuprofen (500 µmol/L) was partially reversed by simultaneous perfusion of 100 nmol/L of carbacyclin and completely reversed by 1 µmol/L of carbacyclin.

Pressure in the fetal afferent portion of the perfusion system was continuously monitored in all experiments. In 12 of 14 experiments, fetal afferent pressure increased 1 to 3 mm Hg during perfusion of cyclooxygenase inhibitors and returned to baseline values during the washout period (with the exception of the indomethacin-treated preparations in which pressure remained elevated after washout). In the remaining two experiments, fetal afferent pressure remained unchanged throughout the experiment. In addition, simultaneous perfusion of carbacyclin with ibuprofen resulted in a dosage-related return of increased pressure to baseline values.

Comment

The results of the present study suggest that prostacyclin may be an important mediator of placental transfer through its action as a vasodilator in the placental vascular bed. In the dual-perfused human placental lobe, a decrease in prostacyclin synthesis reduced diffusional transfer, as measured by the clearance of the freely diffusible substance antipyrine and in most cases increased fetal afferent pressure. A role for prostacyclin in placental transfer is further strengthened by the observation that its analogue, carbacyclin, reversed the effects of cyclooxygenase inhibition on antipyrine clearance. Although antipyrine is a weak inhibitor of cyclooxygenase ($IC^{50} = 4.5$ mmol/L versus cyclooxygenase with arachidonic acid as substrate[13]), the lower concentration used in our experiments (0.16 mmol/L) had no such effect on 6-keto-PGF$_{1\alpha}$ production.

Prostacyclin is produced by endothelial cells of umbilical vessels and may play a major role in the relaxation of umbilical vessels and the prevention of platelet adherence to intact endothelium. The synthesis of prostacyclin is significantly related to umbilical blood flow, as measured by ultrasonography in pregnant women and subsequent superfusion of the umbilical arteries[14] but not to intervillous blood flow, as measured by the xenon-133–isotope method.[15] Prostacyclin synthesis is reduced in the umbilical circulation of patients with severe preeclampsia, IUGR, and chronic hypertension,[1] another indication that this metabolite is involved in the regulation of umbilical blood flow. However, the role of prostacyclin in the circulation of the placental vascular bed, particularly in the capillaries of the terminal villi, has not yet been determined partially because of the inability to study the in vivo situation and the difficulty in separating placental cell types in vitro.

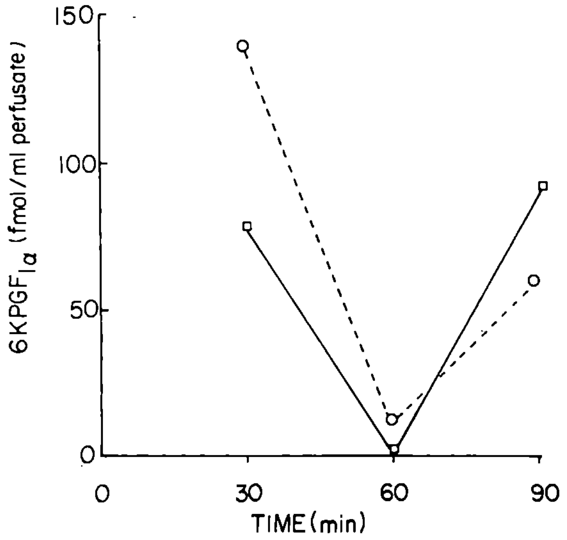

Fig. 2. Effect of cyclooxygenase inhibitors on 6-keto-prostaglandin F$_{1\alpha}$ production in the fetal placental vasculature. Levels of 6-keto-PGF$_{1\alpha}$ in fetal effluent were determined by specific radioimmunoassay after a 30-minute baseline period, following a 30-minute perfusion with 100 µmol/L of indomethacin (o - - - - o) or 500 µmol/L of ibuprofen (□——□), and following a final 30-minute washout period.

The results presented here are an initial insight into what effect the reduction in synthesis of a mediator of vascular dynamics in the maternal-fetal exchange area may have on the movement of nutrients to the developing fetus. Our results suggest that the transfer of substances across the placenta by simple diffusion (oxygen, carbon dioxide, steroids, nucleosides, some electrolytes, fat-soluble vitamins, fatty acids) may be reduced as a result of decreased prostacyclin synthesis. However, a reduction in fetal blood flow through the maternal-fetal exchange area may also reduce the movement of substances transferred by other mechanisms (for example, facilitated diffusion, active transport). This possibility is presently under study. In addition, other vasoactive prostanoids (such as PGD_2) and nonprostanoid agents (angiotension II, bradykinin, histamine) also may be involved in vascular dynamics in the placenta, and the interaction of these substances may confer a native vascular tone.

The results of experiments demonstrating that the prostacyclin analogue, carbacyclin, can reverse the effects of cyclooxygenase inhibition serve to strengthen the suggestion that prostacyclin plays a role in the regulation of placental transfer. These results may have implications for the treatment of placental insufficiency. Although two recent studies[3, 4] have failed to demonstrate an effect on placental blood flow after infusion of prostacyclin in normal patients or patients with preeclampsia, these studies monitored only intervillous and umbilical blood flow and therefore may have been unable to detect important changes in flow through the placental capillary bed. Because vascular shunts are present in the capillary network of placental villi, it may be possible to redirect fetal blood flow past the maternal-fetal exchange area, thereby reducing placental transfer without significant effects on umbilical blood flow. These studies used relatively unstable prostacyclin in an attempt to alter placental blood flow, whereas a stable analogue may prove to be more effective. In addition, maternally-administered prostacyclin may not exert effects in the placental vascular bed, since it may not cross the placenta. However, preliminary results from our laboratory (we used gas chromatography–mass spectrometry analysis of carbacyclin in fetal effluent) suggest that carbacyclin is readily detectable in fetal effluent after administration in the maternal afferent circulation.

Finally, the effects of indomethacin and ibuprofen on prostacyclin synthesis and placental transfer demonstrated in this study reconfirm the need for caution in the use of cyclooxygenase inhibitors during pregnancy. Several of these substances readily cross the placenta[16] and have been shown to inhibit prostacyclin synthesis in the umbilical cord. In pathologic pregnancies in which prostacyclin synthesis is reduced relative to that of thromboxane A_2, inhibition of cyclooxygenase may be beneficial in reducing thromboxane A_2 production and thereby restoring a balance between these mediators of vasodilation and vasoconstriction. However, our studies suggest that the inhibition of cyclooxygenase may reduce placental transfer and may therefore compromise the developing fetus. Furthermore, these studies present the possibility that prostacyclin analogues may increase placental transfer in situations in which prostacyclin synthesis and placental blood flow are reduced and therefore may serve as an effective therapeutic approach in the treatment of pathologic pregnancies.

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REFERENCES

1. Stuart MJ, Sunderji SG, Yambo T, et al. Decreased prostacyclin production: a characteristic of chronic placental insufficiency syndromes. Lancet 1981;i:1126-8.
2. Wallenburg HCS, Rotmans N. Enhanced reactivity of the platelet thromboxane pathway in normotensive and hypertensive pregnancies with insufficient fetal growth. AM J OBSTET GYNECOL 1982;144:523-8.
3. Husslein P, Gitsch E, Pateisky N, et al. Prostacyclin does not influence placental blood pool in vivo. Gynecol Obstet Invest 1985;19:78-81.
4. Jouppila P, Kirkinen P, Koivula A, Ylikorkala O. Failure of exogenous prostacyclin to change placental and fetal blood flow in preeclampsia. AM J OBSTET GYNECOL 1985;151:661-5.
5. Aspirin and pre-eclampsia [Editorial]. Lancet 1986;i: 18-20.
6. Setty BNY, Walenga RW, Stuart MJ. Kinetic analysis of the effects of hyperoxia and hypoxia on vascular cyclooxygenase activity in vitro. Biochem Biophys Res Commun 1984;125:170-6.
7. Challier J-C, Schneider H, Dancis J. In vitro perfusion of human placenta V. Oxygen consumption. AM J OBSTET GYNECOL 1976;126:261-5.
8. Haugel S, Challier J-C, Cedard L, Olive G. Metabolism of the human placenta perfused in vitro: glucose transfer and utilization, O_2 consumption, lactate and ammonia production. Pediatr Res 1983;17:729-32.
9. Lands WEM, Hanel AM. Inhibitors and activators of prostaglandin biosynthesis. In: Pace-Asciak C, Granström E, eds. Prostaglandins and related substances. Amsterdam: Elsevier Science Publishers, 1983:203.
10. Brodie BB, Axelrod J, Soberman R, Levy BB. An estimation of antipyrine in biological materials. J Biol Chem 1949;179:25-9.
11. Whittle BJR, Moncada S, Whiting F, Vane JR. Carbacyclin—a potent stable prostacyclin analogue for the inhibition of platelet aggregation. Prostaglandins 1980; 19:605-27.
12. Walenga RW, Wall SF, Setty BNY, Stuart MJ. Indomethacin inhibition of platelet cyclooxygenase is slowly reversible. Prostaglandins 1986;31:625-37.
13. Blackwell GJ, Flower RJ. 1-phenyl-3-pyrazolidine: an inhibitor of cyclooxygenase and lipoxygenase pathways in lung and platelet. Prostaglandins 1978;16:417-25.
14. Mäkilä U-M, Jouppila P, Kirkinen P, et al. Relation between umbilical prostacyclin production and blood flow in the fetus. Lancet 1983;i:728-9.
15. Ylikorkala O, Jouppila P, Kirkinen P, Viinkka L. Maternal prostacyclin, thromboxane, and placental blood flow. AM J OBSTET GYNECOL 1983;145:730-2.
16. Akbaraly R, Leng JJ, Brachet-Liermain, et al. Transplacental transfer of four anti-inflammatory agents. A study carried out by in vitro perfusion. J Gynecol Obstet Biol Reprod (Paris) 1981;10:7-11.

Beneficial effects of U-63,557A, a thromboxane synthetase inhibitor, in an ovine model of pregnancy-induced hypertension

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Pregnancy-induced hypertension was induced in five ewes (gestational day 135; term 150 days) by 72 hours of food deprivation. Maternal arterial pressure, uterine blood flow, platelet function, renal function, and plasma levels of 6-ketoprostaglandin $F_{1\alpha}$ and thromboxane B_2 were measured before and during hypertension and after three intravenous injections of U-63,557A; sodium 5-(3′-pyridinylmethyl) benzofuran-2-carboxylate, monohydrate (30 mg/kg every 8 hours). Blood pressure increased ($p < 0.03$), and returned to normal after U-63,557A. Left uterine artery blood flow increased after U-63,557A ($p < 0.03$). Creatinine clearance decreased during hypertension ($p < 0.03$) and increased after U-63,557A. Urine protein increased during hypertension ($p < 0.03$) and decreased after treatment. Platelet count dropped during hypertension ($p < 0.03$) and was elevated after treatment. Collagen lag phase decreased during hypertension ($p < 0.03$) and increased after treatment. After U-63,557A, 6-ketoprostaglandin $F_{1\alpha}$ levels were higher ($p < 0.04$) than baseline or hypertensive values. Administration of a thromboxane synthetase inhibitor caused resolution of hemodynamic, renal, and coagulation dysfunctions that occurred in ovine pregnancy-induced hypertension. (AM J OBSTET GYNECOL 1987;157:199-203.)

Key words: Pregnancy-induced hypertension, U-63,557A, thromboxane synthetase inhibitor, sheep

Key pathophysiologic roles for prostacyclin deficiency[1-3] and/or thromboxane A_2 excess[4, 5] have been suggested in pregnancy-induced hypertension. Recently we described an ovine model of pregnancy-induced hypertension,[6] and we have reported preliminary data that document beneficial effects of prostacyclin in our model.[7] In this study we investigated the effects of U-63,557A, sodium 5-(3′-pyridinylmethyl) benzofuran-2-carboxylate, monohydrate, a selective thromboxane synthetase inhibitor, in five gravid ewes in which hypertension had been induced.

Material and methods

Eight pregnant ewes were obtained and conditioned to metabolism crates. At gestational days 125 to 128 (term 150 days), the ewes were sedated with intravenous pentobarbital (6 mg/kg) and placed in a right lateral recumbent position. General anesthesia was induced and maintained with 1.5% methoxyflurane and oxygen was delivered via an endotracheal tube. A Swan-Ganz thermal dilution catheter (Elecath) was placed percutaneously in the pulmonary artery via the external jugular vein for measurement of cardiac output, and a catheter was placed in the descending aorta via the carotid artery for arterial pressure measurement. A laparotomy was performed in the left paralumbar fossa, and a square-wave electromagnetic flow probe (Biotronex Laboratory, Inc.) was placed around the left uterine artery for qualitative measurement of uterine blood flow. Three ewes carried single lambs in the left horn of the uterus. The laparotomy incision was then closed, and anesthesia was discontinued. Catheters were affixed to the neck with a bandage and were flushed daily with heparinized saline solution. All ewes received 10 mg/kg ampicillin three times a day intramuscularly for 3 days.

Two ewes that were carrying triplets died within 2 days of operation of fulminating eclampsia induced by the stress of the surgical procedure. This is consistent with the disease as seen in clinical veterinary practice, in that ewes carrying more than two fetuses are extremely susceptible to fulminating eclampsia and death if placed under stress. To date, the only effective treat-

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Table I. Maternal mean arterial pressure and left uterine artery blood flow (mean ±SE)

	Baseline	*Hypertension*	*Posttreatment*
Mean arterial pressure (mm Hg)	92.24 ± 2.05	108.33 ± 2.95*	88.33 ± 5.40
Left uterine artery blood flow (ml/min/lamb)	236.95 ± 32.65	260.25 ± 55.85	320.31 ± 54.26*

$^*p < 0.03$.

Table II. Creatinine clearance and urine protein (mean ± SE)

	Baseline	*Hypertension*	*Posttreatment*
Creatinine clearance (ml/min/kg)	1.46 ± 0.10	0.71 ± 0.16*	1.09 ± 0.23
Urine protein (mg/dl)	40.94 ± 18.50	62.86 ± 18.90*	34.17 ± 16.54

$^*p < 0.03$.

ment for ovine toxemia of pregnancy has been prompt and immediate delivery of the fetuses, and this always results in fetal death due to prematurity. Since the clinical course is almost invariably fatal to both mother and fetuses if delivery is not accomplished, each animal served as its own control. No ewes were deprived of food to induce hypertension and then not treated.

After allowing approximately 3 to 7 days for postsurgical stabilization, baseline recordings of the following parameters were taken every 12 hours during a 48-hour period in six instrument-manipulated ewes, beginning on gestational day 135: (1) maternal mean arterial pressure, (2) cardiac output, (3) total peripheral resistance, and (4) left uterine artery blood flow. Blood samples were taken for determination of serum creatinine, blood urea nitrogen, electrolytes, total protein, serum glutamic oxaloacetic transaminase, and glucose. Urine was sampled for determination of protein concentration. Samples were analyzed in the clinical pathology laboratory of the Virginia-Maryland Regional College of Veterinary Medicine Veterinary Teaching Hospital. Jugular blood samples (4.5 ml) were collected into 0.5 ml of cold 3.8% sodium citrate and 50 μg of indomethacin, centrifuged at 3000 × g for 15 minutes, and then frozen at −70° C until analyzed for thromboxane B_2 and 6-ketoprostaglandin $F_{1\alpha}$ by radioimmunoassay.

The levels of 6 ketoprostaglandin $F_{1\alpha}$ and thromboxane B_2 were determined from 300 μl aliquots of unextracted plasma by the use of Amersham commercial radioimmunoassay kits. Highly specific radioimmunoassays, which consisted of competitive binding of radioactive and natural thromboxane B_2 or 6-ketoprostaglandin $F_{1\alpha}$ to a highly specific antibody, were used. Dextran-coated charcoal was used to separate the bound from the free fraction. The least detectable concentrations of 6-ketoprostaglandin $F_{1\alpha}$ and thromboxane B_2 were 5 pg per tube. Between-assay variation for both compounds was ≤10%, and within-assay variation for both was ≤5%. Fifty percent binding of the standard curves was 51.33 pg for 6-ketoprostaglandin $F_{1\alpha}$ and 27 pg for thromboxane B_2.

Another venous blood sample (9.0 ml) was drawn into 1 ml of 3.2% sodium citrate, and platelet counts and platelet aggregation testing were performed manually and in a Payton dual-channel platelet aggregometer, respectively. Complete platelet aggregation studies are reported elsewhere.[8] After the platelet blood samples were drawn, the syringes were gently inverted several times for adequate distribution of the citrate, and then the samples were transferred to capped polypropylene tubes. A 20 μl sample was taken from each tube for determination of the whole-blood platelet count. The tubes were allowed to sit at room temperature for 20 minutes and then centrifuged at 150 × g for 12 minutes; platelet-rich plasma was collected. Platelet-poor plasma was derived by centrifugation at 4000 × g for 15 minutes. The platelet-rich plasma platelet counts were adjusted to 250,000/mm^3 by the addition of platelet-poor plasma, and the platelet aggregation response to collagen (Bio/Data Corporation) was assessed. The platelet-rich plasma was stirred at 800 rpm for 2 minutes, and collagen was then added at a final concentration of 0.19 mg/ml. The time from collagen addition until platelet aggregation began was measured in seconds (collagen lag phase) and used as an indicator of platelet sensitivity and reactivity.

Glomerular filtration rate was estimated by an exogenous creatinine clearance procedure. Creatinine was administered intravenously at a rate of 400 mg/min for 10 minutes. After a steady-state plasma concentration of creatinine (15 mg/dl) was achieved, the rate was lowered to 200 mg/min and a 20-minute urine collection was taken via a Foley catheter placed

Table III. Platelet counts and collagen lag phase (mean ± SE)

	Baseline	*Hypertension*	*Posttreatment*
Platelet count ($\times 1000/mm^3$)	316.17 ± 20.9	230.60 ± 25.75*	313.20 ± 37.56
Collagen lag phase (second)	30 ± 4.75	9.6 ± 5.45*	46.8 ± 20.94

*$p < 0.03$.

Table IV. Selected serum biochemical values (mean ± SE)

	Baseline	*Hypertension*	*Posttreatment*
Glucose (mg/dl)	49.14 ± 6.28	30.32 ± 3.49	40.18 ± 4.95
Blood urea nitrogen (mg/dl)	22.11 ± 3.78	23.3 ± 4.29	34.43 ± 5.13
Creatinine (mg/dl)	1.72 ± 0.30	1.90 ± 0.24	2.41 ± 0.27

in the bladder. Creatinine clearance was calculated as previously described.[6]

After these baseline studies, ovine pregnancy-induced hypertension was induced by 72 hours of food deprivation, and selected parameters were monitored every 12 hours. When the clinical signs were manifest, complete recordings and testing were repeated. Pregnancy-induced hypertension was defined as: (1) hypertension, a mean arterial pressure elevation of >15 mm Hg; (2) significantly increased urine protein; (3) decreased glomerular filtration rate and uterine blood flow; (4) depression and/or seizures. Hypertension was induced in five of the six ewes by 72 hours of food deprivation. The sixth ewe did not develop any clinical or biochemical signs of toxemia of pregnancy after 5 days of food deprivation. Necropsy revealed that this ewe carried only one lamb.

The five ewes were treated with U-63,557A (30 mg/kg every 8 hours), administered intravenously (gestational day 140). U-63,557A was obtained in crystalline form from The Upjohn Company and dissolved in 0.9% saline solution at a final concentration of 200 mg/ml. It was stored at 4°C and administered in a bolus fashion. Treatment continued for 24 hours. Eight hours after the third intravenous injection, complete recordings and testing were repeated. The ewes were then allowed to feed and observed until lambing time.

Each animal served as its own control and statistical analysis was accomplished by paired t test comparisons and an analysis of variance. A p value of <0.05 was considered significant.

Results

Table I summarizes changes in selected hemodynamic parameters measured in this study. Hypertension occurred after as little as 24 hours of food deprivation in three of five ewes and by 72 hours in the two remaining ewes. The three ewes that manifested hypertension quickly carried twins, while the other three ewes carried single lambs. Mean arterial pressure increased 18.3% and was significantly different from baseline and posttreatment levels ($p < 0.03$). In this study, left uterine artery blood flow did not change significantly with the onset of hypertension. However, after three doses of U-63,557A, left uterine blood flow was increased 35% over baseline values ($p < 0.03$).

Renal dysfunction (Table II) occurred with the onset of hypertension and resulted in a 51% decrease in glomerular filtration rate, estimated by creatinine clearance ($p < 0.03$). After administration of U-63,557A, glomerular filtration rate increased 25% and was not significantly different from baseline values ($p > 0.05$). Concurrent with the decrease in renal function, urine protein levels rose 54% ($p < 0.03$). Administration of U-63,557A reduced urine protein secretion to baseline levels.

Platelet function was altered during hypertension (Table III). Platelet counts dropped 27% with the onset of hypertension ($p < 0.03$) and returned to normal after treatment with the thromboxane synthetase inhibitor. Platelet sensitivity to collagen activation increased during toxemia ($p < 0.03$) and was normalized by administration of the thromboxane synthetase inhibitor.

Less dramatic changes occurred in biochemical parameters that were studied (Table IV). Food deprivation caused a drop in glucose levels, which then recovered somewhat after drug administration but before feeding. However, none of the changes was significant. Both blood urea nitrogen and creatinine tended to rise after drug administration but these changes were not significant.

Plasma 6-ketoprostaglandin $F_{1\alpha}$ and thromboxane B_2 levels are shown in Table V. At baseline the ratio of 6-ketoprostaglandin $F_{1\alpha}$ to thromboxane B_2 in plasma was 1:2.25. Since both 6-ketoprostaglandin $F_{1\alpha}$ and thromboxane B_2 are eliminated via glomerular filtration, er-

Table V. Plasma 6-ketoprostaglandin $F_{1\alpha}$ and thromboxane B_2 levels (mean ± SE)

	Baseline	*Hypertension*	*Posttreatment*
6-Ketoprostaglandin $F_{1\alpha}$ (pg/ml)	48.06 ± 6.93	35.66 ± 18.73	96.77 ± 26.16*
Thromboxane B_2 (pg/ml)	108.08 ± 13.50	68.1 ± 17.78	80.71 ± 25.8

*Higher than baseline or hypertensive values, $p < 0.04$.

roneously high levels would be reported if the decreased glomerular filtration rate was not considered. Therefore at the time of hypertension and after treatment the raw values for these parameters were transformed with the use of the ratio of the glomerular filtration rate during hypertension or after treatment to the baseline glomerular filtration rate, thereby negating recirculation of the compounds due to lowered clearances. During hypertension both 6-ketoprostaglandin $F_{1\alpha}$ and thromboxane levels tended to decrease, but neither reduction was significant. Eight hours after the last dose of U-63,557A, thromboxane B_2 levels were not significantly different from baseline or hypertensive levels. However, 6-ketoprostaglandin $F_{1\alpha}$ levels had increased 101% and were higher than baseline and hypertension values ($p < 0.04$).

Treatment with U-63,557A appeared to improve the neurological function of the ewes, which were depressed and exhibited generalized muscle tremors before drug administration. After treatment, they became more responsive, alert, and muscle tremors disappeared.

Four ewes delivered live lambs at day 148, and one ewe, which died of a cerebral arterial thrombus (a complication from the arterial pressure line), was judged to be carrying a live single lamb at the time of death. In this study, fetal monitoring was not accomplished, but the lambs were carefully observed at birth for any adverse effects. All were vigorous, healthy, and continue to thrive. Current studies underway in our laboratory have included fetal heart rate monitoring, and signs of fetal distress noted during hypertension have resolved after U-63,557A administration (work in progress).

Comment

The administration of U-63,557A was beneficial in terms of correcting hemodynamic, coagulation, and renal dysfunctions in this ovine model. The observations concerning decreased mean arterial blood pressure and concurrent increased left uterine artery blood flow were of particular interest. Some researchers, using sheep as a model for uterine blood flow, believe that the uterine arteries function as maximally dilated conduit vessels and that sudden drops in arterial driving pressure might decrease uteroplacental perfusion.[9] However, we saw increased left uterine artery blood flow when blood pressure was reduced. This increase may indicate that uteroplacental resistance was decreased in response to thromboxane synthetase inhibition. However, we did not measure total uteroplacental blood flow or resistance, and thus this is only indirect evidence.

After treatment, renal function returned to normal levels and the urine output of the ewes remained good throughout subsequent pregnancy. The values for glomerular filtration rate in chronically instrumented pregnant ewes that we report are lower than those previously reported.[10] We also report a more profound decrease in glomerular filtration rate than reported in earlier studies of ovine pregnancy toxemia.[10] Serum creatinine levels tended to rise slightly, but not significantly, after treatment. This may have been in response to serial creatinine clearance procedures performed 24 hours apart or may have been in response to increased muscle catabolism during food deprivation. Urine protein increased as glomerular filtration rate decreased and then decreased after therapy with U-63,557A. Although these changes were significant, additional studies are needed to determine normal urine protein levels in pregnant sheep.

Platelet[11, 12] and coagulation[13] abnormalities occur in women with pregnancy-induced hypertension, and marked platelet changes occurred in the present studies. Enhancement of thromboxane pathway activity in platelets has been observed in placental insufficiency syndromes such as pregnancy-induced hypertension.[14] It may have been present in our sheep since administration of the thromboxane synthetase inhibitor increased platelet counts and normalized in vitro platelet function. Although it is tempting to suggest that thromboxane A_2 excess may be crucial in the development of the platelet and hemodynamic derangements in this model, we did not see sharp increases in thromboxane B_2 levels. Instead we report a slight, but insignificant, decrease in both thromboxane B_2 and 6-ketoprostaglandin $F_{1\alpha}$ levels during hypertension. Even more interesting is the fact that 8 hours after the third dose of U-63,557A, thromboxane B_2 levels were not different from baseline or hypertension values, but 6-ketoprostaglandin $F_{1\alpha}$ levels were increased 101% over baseline values, thus supporting the concept of shunting substrate from thromboxane synthetase to prostacyclin synthetase via thromboxane synthetase inhibition.

One likely possibility for the pathophysiologic mechanism that induces our model of pregnancy-induced hypertension may be the development of an imbalance in the prostacyclin : thromboxane A_2 ratio caused by the ketonemia that develops in food-deprived, gravid ewes.[6, 10] Increased levels of circulating free fatty acids have been reported to decrease prostacyclin survival time in human plasma,[15] and this may be occurring in our model. Retrograde infusion of β-hydroxybutyrate into the uterine arteries of gravid ewes has decreased uterine blood flow.[16] The imbalance induced by ketonemia may initiate a positive feedback cycle that then maintains and intensifies the prostacyclin : thromboxane A_2 imbalance as the result of further reductions in uteroplacental perfusion.

In summary, infusion of U-63,557A in sheep with ovine pregnancy-induced hypertension resulted in amelioration of coagulation, hemodynamic, renal, and neurological clinical signs. The indirect mechanism for this action seemed to be the result of a 101% increase in prostacyclin levels. The exact mechanism of this phenomenon remains to be elucidated.

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REFERENCES

1. Dadak C, Kefalides A, Sinzinger H, Weber G. Reduced umbilical artery prostacyclin formation in complicated pregnancies. AM J OBSTET GYNECOL 1982;144:792.
2. Yamaguchi M, Mori N. 6-Keto prostaglandin $F_{1\alpha}$, thromboxane B_2, and 13,14-dihydro-15-keto prostaglandin F concentrations of normotensive and preeclamptic patients during pregnancy, delivery, and the postpartum period. AM J OBSTET GYNECOL 1985;151:121.
3. Makila U, Viinikka L, Ylikorkala O. Evidence that prostacyclin deficiency is a specific feature in preeclampsia. AM J OBSTET GYNECOL 1984;148:772.
4. Walsh SW. Preeclampsia: an imbalance in placental prostacyclin and thromboxane production. AM J OBSTET GYNECOL 1985;152:335.
5. Makila UM, Viinikka L, Ylikorkala O. Increased thromboxane A_2 production but normal prostacyclin by the placenta in hypertensive pregnancies. Prostaglandins 1984;27:87.
6. Thatcher CD, Keith JC Jr. Pregnancy-induced hypertension: development of a model in the pregnant sheep. AM J OBSTET GYNECOL 1986;155:201.
7. Keith JC Jr, Thatcher CD. Ovine pregnancy toxemia: renal changes induced by prostacyclin infusion [Abstract No. 312]. In: Proceedings of the sixty-fifth annual meeting of the Conference of Research Workers in Animal Disease, Chicago, Illinois, November 12-13, 1984.
8. Keith JC, Schaub RG, Thatcher CD. U-63,557A normalizes in vitro platelet aggregation in an ovine model of pregnancy-induced hypertension. Thromb Res (in press).
9. Lindheimer MD, Katz AI. Hypertension and pregnancy. In: Genest J, Kerchel O, Hamet Pand Cantin M, eds. Hypertension. New York: McGraw-Hill, 1983:889.
10. Kaufman CF, Bergman EN. Renal function studies in normal and toxemic pregnant sheep. Cornell Vet 1978;68:124.
11. Weinstein L. Syndrome of hemolysis, elevated liver enzymes, and low platelet count: a severe consequence of hypertension in pregnancy. AM J OBSTET GYNECOL 1982;142:159.
12. Morrison R, Crawford J, MacPherson M, Heptinstall S. Platelet behavior in normal pregnancy, pregnancy complicated by essential hypertension and pregnancy-induced hypertension. Thromb Haemost 1985;54:607.
13. Vaziri ND, Toohey J, Powers D, et al. Activation of intrinsic coagulation pathway in pre-eclampsia. Am J Med 1986;80:103.
14. Wallenburg HCS, Rotmans N. Enhanced reactivity of the platelet thromboxane pathway in normotensive and hypertensive pregnancies with insufficient fetal growth. AM J OBSTET GYNECOL 1982;144:523.
15. Goodnight SH, Inkeles SB, Kovach NL, Connor WE. Reduced prostacyclin survival after fasting-induced elevation of plasma free fatty acids. Thromb Haemost 1985;54:418.
16. Miodovnik M, Lavin JP, Harrington DJ, Leung LS, Seeds AE, Clark KE. Effects of maternal ketoacidemia on the pregnant ewe and the fetus. AM J OBSTET GYNECOL 1982;144:585.

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Disposition of acute, multiple-dose ethanol in the near-term pregnant ewe

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The disposition of ethanol and its proximate metabolite, acetaldehyde, was determined in seven conscious instrumented pregnant ewes (127 to 132 days of gestation; term, 147 days) for intravenous infusion of four dosages of 0.5 gm ethanol/kg maternal body weight, administered over 5 hours to the mother. The maternal and fetal blood had ethanol concentrations that were maximal at 5 hours and were virtually identical during the 24-hour study. There was delayed transfer of ethanol into the amniotic and allantoic fluids during the dosing period, followed by higher ethanol concentrations in these fluids during the elimination phase compared with fetal blood. The ethanol elimination rate was similar for the four biologic fluids. Acetaldehyde concentrations in the four fluids were a thousandfold less than the respective ethanol concentrations. The maternal blood acetaldehyde concentration was greater than that in fetal blood. The data indicate that for a binge-type drinking episode during near-term pregnancy, there is unimpeded bidirectional placental transfer of ethanol between the mother and the fetus; the amniotic fluid surrounding the fetus is a reservoir for ethanol in utero; elimination of ethanol from the maternal-fetal unit is regulated by maternal hepatic biotransformation of ethanol; and there is appreciable acetaldehyde-oxidizing capacity in the maternal liver and at extrahepatic sites. (AM J OBSTET GYNECOL 1987;157:204-11.)

Key words: Multiple-dose ethanol, acetaldehyde, disposition, pregnant ewe

Ethanol ingestion during pregnancy can produce a wide range of effects on the developing embryo-fetus. Epidemiologic studies have established that chronic consumption of ethanol during pregnancy is teratogenic (fetal alcohol syndrome) and increases the risk of spontaneous abortion.[1, 2] The neonatal growth retardation observed in the fetal alcohol syndrome[1] and the incidence of spontaneous abortion[2] appear to be dose-related. It has been reported[3] that ingestion of a single low dose of ethanol by women in the third trimester of pregnancy decreased fetal breathing movements without affecting fetal heart rate and gross body movements.

Studies of the mechanisms of the teratogenic and fetotoxic effects of maternal ethanol ingestion have included the investigation of the pharmacokinetics of ethanol in the maternal-fetal unit. Most studies to date have focused on the pharmacokinetics of a single low dose of ethanol administered to near-term pregnant experimental animals. These investigations have demonstrated similar ethanol concentrations and kinetics of ethanol elimination for the mother and the fetus[4-7] and differential disposition of ethanol in amniotic fluid,[4, 7] which may serve as a reservoir for ethanol in utero, thereby providing a second route of fetal exposure to ethanol.

It has not been established whether the pharmacokinetics of ethanol during pregnancy are dose-related. However, it has been reported[8] that for intravenous bolus infusion of high-dose ethanol into the anesthetized pregnant monkey, the ethanol concentration in fetal blood was about one third that in maternal blood. These data indicate that the pharmacokinetics of low-dose and high-dose ethanol may be different.

It is controversial whether acetaldehyde, the proximate metabolite of ethanol, plays a role in the fetal alcohol syndrome and ethanol-induced fetotoxicity.[9, 10] Technical difficulties involving artifactual acetaldehyde formation during sample analysis have limited the investigation of the pharmacokinetics of ethanol-derived acetaldehyde and its possible involvement in the fetal effects of maternal ethanol administration.

In view of the incomplete elucidation of the dispo-

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sition of ethanol during pregnancy for different dosage regimens, the objective of the present study was to determine the pharmacokinetics of ethanol and its metabolite, acetaldehyde, in the maternal-fetal unit for administration of high-dosage ethanol to the mother, given as multiple dosages on one day, to mimic a binge-type drinking episode. This investigation was conducted in the conscious instrumented near-term pregnant ewe, which has been used extensively in studies of the pharmacokinetics of drugs in the maternal-fetal unit,[11] and an analytical procedure that minimizes artifactual acetaldehyde formation was used to measure ethanol-derived acetaldehyde concentrations in the biologic fluids.[12]

Material and methods

Experimental animals. All animals were cared for in compliance with the principles and guidelines of the Canadian Council on Animal Care. Experiments were performed on seven dated mixed-breed pregnant ewes and their fetuses between 127 and 132 days of gestation (term, 147 days). Three ewes had singleton pregnancies, and four ewes had twins. The singleton fetus or one of the twins of each ewe was surgically prepared for study at least 4 days before experimentation. Anesthesia was induced in each ewe by intravenous administration of 40 mg of sodium thiopental; after intubation, anesthesia for surgery was maintained with 1.5% to 2% halothane and a 1:1 mixture of nitrous oxide and oxygen at a flow rate of 1 L/min with closed-circuit ventilation. Under sterile conditions, the uterus was exposed and opened through a lower abdominal midline incision. The fetus was partially exteriorized during the surgical instrumentation. Electrodes of stainless steel Teflon-coated wire within polyvinyl catheters were implanted biparietally on the dura for electrocorticographic recording, through the orbital ridge of the zygomatic bone of each eye for electrooculographic recording and over the sternum for fetal heart rate recording. A reference electrode was placed in the loose connective tissue overlying the occipital bone of the skull. Polyvinyl catheters were placed in the sagittal sinus, the brachiocephalic artery via a forelimb axillary artery, the inferior vena cava via a hindlimb tarsal vein, and a hindlimb femoral artery. A catheter was placed in the trachea, and two catheters were placed in the amniotic cavity and secured to a fetal forelimb and hindlimb. The fetus was repositioned in the amniotic cavity, and the uterus was closed. A catheter was inserted into the allantoic sac, which surrounds the amniotic sac, via a small incision in the uterine horn, and was secured with a purse-string suture. The catheters, filled with heparinized saline solution, and the electrodes were exteriorized through the flank of the ewe, and the abdomen was closed in layers. Polyvinyl catheters then were placed in a maternal femoral artery and femoral vein. At the time of surgery, 1 gm of dihydrostreptomycin and 800,000 units of penicillin G were injected intramuscularly into the ewe. After the uterus was closed, 1 million units of penicillin G was injected into the fetal tarsal vein and amniotic cavity. To control pain after surgery, 100 mg of meperidine was administered intramuscularly to the ewe. Administration of antibiotics was continued daily for 3 days. After surgery the ewes were housed in individual cages suitable for continuous monitoring and were provided with food and water ad libitum during the recovery period and experimentation. All catheters were flushed daily. This experimental animal preparation was used to study simultaneously the pharmacokinetics and pharmacodynamics of ethanol in the maternal-fetal unit. The effects of maternal ethanol administration on fetal breathing activity, brain and cardiovascular function, acid-base status, cerebral metabolism, and organ blood flow will be reported separately.

Experimental protocol. Four dosages of 0.5 gm ethanol/kg maternal body weight were infused into the maternal vena cava of each ewe over a 5-hour period. Each dosage, as a 40% volume of solute per volume of solvent solution (Alcohol, Liquor Control Board of Ontario), was administered by an infusion pump over 0.5 hour, and there was a 1-hour interval between each dosage to allow extravascular distribution of ethanol in the maternal and fetal compartments before each succeeding dosage.[7] The ethanol dosage regimen was initiated between 9:30 and 11:00 AM. A 0.25 ml aliquot of each of the following: maternal arterial blood from the maternal femoral artery, fetal arterial blood from the fetal brachiocephalic artery, amniotic fluid, and allantoic fluid was collected before ethanol infusion (0 hour), during the 5-hour period of administration of the four ethanol dosages (0.5, 1.5, 2.0, 3.0, 3.5, 4.5, 5.0 hours), and hourly thereafter until 24 hours. All seven ewes were studied for 24 hours. Because of difficulties in maintaining patency of the catheter in the allantoic cavity, reliable monitoring of the allantoic fluid for 24 hours was only possible in three ewes. All samples were analyzed for ethanol and acetaldehyde on the day of experimentation.

Analysis of ethanol and acetaldehyde. A headspace gas-liquid chromatographic procedure requiring 100 μl of sample was used for the quantitation of ethanol and acetaldehyde, in which artifactual acetaldehyde formation was minimized during sample preparation.[12] The overall accuracy of the procedure was 99.5% ± 1.1% (SE) for ethanol and 105.3% ± 3.3% (SE) for acetaldehyde, and the within-day coefficient of variation of the method did not exceed 6.8% for ethanol and 3.9% for acetaldehyde. The lower limit of quantitative sensitivity was 0.005 mg/ml ethanol and 0.10

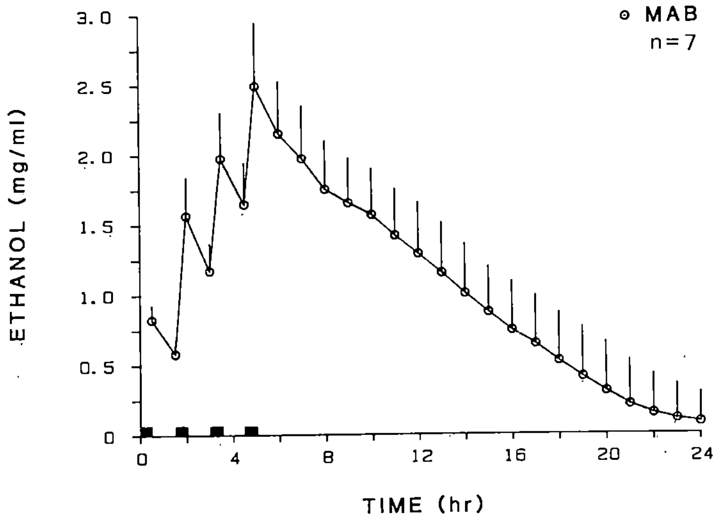

Fig. 1. Ethanol concentration–time curve for maternal arterial blood *(MAB)* of the near-term pregnant ewe (n = 7) during and after maternal administration of ethanol. The data are expressed as the mean ± SD. Four dosages of 0.5 gm ethanol/kg maternal body weight (■) were administered by maternal intravenous infusion over 5 hours, with each dosage infused over 0.5 hour followed by a 1-hour interval before the next dosage.

μg/ml acetaldehyde. The measured acetaldehyde concentrations were corrected for any artifactual acetaldehyde formation during sample preparation and gas-liquid chromatographic analysis as described previously.[12] All samples were analyzed in duplicate.

Data analysis. The ethanol and acetaldehyde concentrations and pharmacokinetic data are presented as the mean ± SD. The kinetic parameters, maximum initial velocity (V_{max}, milligrams × milliliters^{-1} × hours^{-1}), and Michaelis constant for ethanol (K_M, milligrams per milliliter) for in vivo biotransformation of ethanol were calculated from the maternal arterial blood ethanol concentration–time curve of each animal. The data were fitted to the one-compartment open model with intravenous infusion and Michaelis-Menten elimination kinetics using the pharmacokinetic analysis program PCNONLIN (Statistical Consultants, Inc., Lexington, Kentucky) with the ethanol concentrations weighted according to $\frac{1}{(\text{ethanol concentration})^2}$. For each animal the ethanol elimination rate (milligrams × milliliters^{-1} × hours^{-1}) of the apparent linear portion of the elimination phase of each of the maternal arterial blood, fetal arterial blood, amniotic fluid, and allantoic fluid ethanol concentration–time curves was determined from the slope of the line of best fit, calculated by least-squares linear regression analysis. Statistical comparison of the ethanol (and acetaldehyde) concentration data for maternal arterial blood, fetal arterial blood, amniotic fluid, and allantoic fluid was conducted at each time interval by repeated measures, one-way analysis of variance, followed by Newman-Keuls test if a significant F ratio was obtained ($p < 0.05$). The ethanol elimination rate data for maternal arterial blood, fetal arterial blood, amniotic fluid, and allantoic fluid were compared by one-way analysis of variance. The maximum velocity and ethanol elimination rate of the apparent linear phase of the maternal arterial blood ethanol concentration–time curve were compared by Student's *t* test for paired data (two-tailed). Two groups of data were considered to be statistically different when $p < 0.05$.

Results

The mean ethanol concentration–time curve for maternal arterial blood of seven near-term pregnant ewes during and after maternal administration of four doses of 0.5 gm ethanol/kg maternal body weight is presented in Fig. 1. The maternal arterial blood ethanol concentration was maximal (2.492 ± 0.456 mg/ml) at the end of the 5-hour period of ethanol administration and then decreased rapidly during the 5- to 8-hour interval, which indicated extravascular distribution of ethanol from the maternal systemic circulation. During the 8- to 24-hour interval, the maternal arterial blood ethanol concentration decreased more slowly, and this elimination phase was characterized by an apparent linear phase and a terminal curvilinear phase, which was consistent with Michaelis-Menten kinetics of ethanol elimination.[13] Because hepatic biotransformation is the major route of ethanol elimination in adult mammalian species,[14] the kinetic parameters, maximum velocity and Michaelis constant, for in vivo biotransformation of ethanol were calculated from the maternal

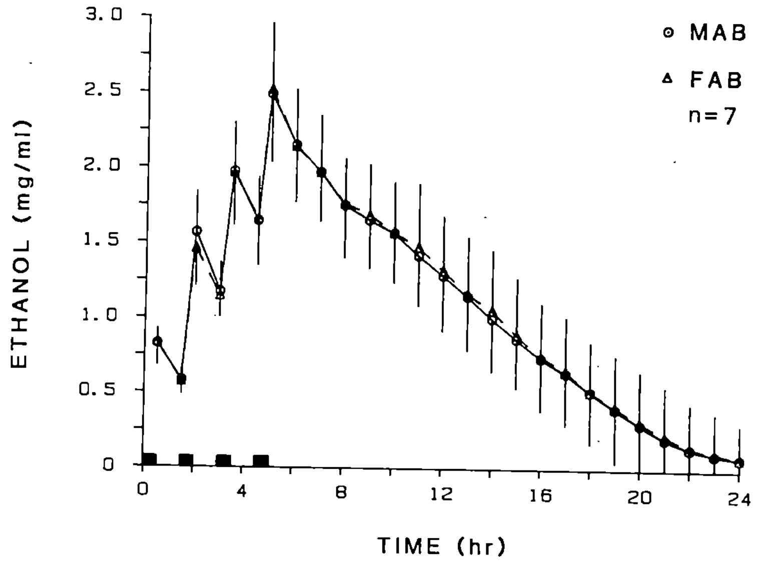

Fig. 2. Ethanol concentration–time curves for maternal arterial blood *(MAB)* and fetal arterial blood *(FAB)* of the near-term pregnant ewe (n = 7) during and after maternal administration of ethanol. Other denotations are described in the legend to Fig. 1.

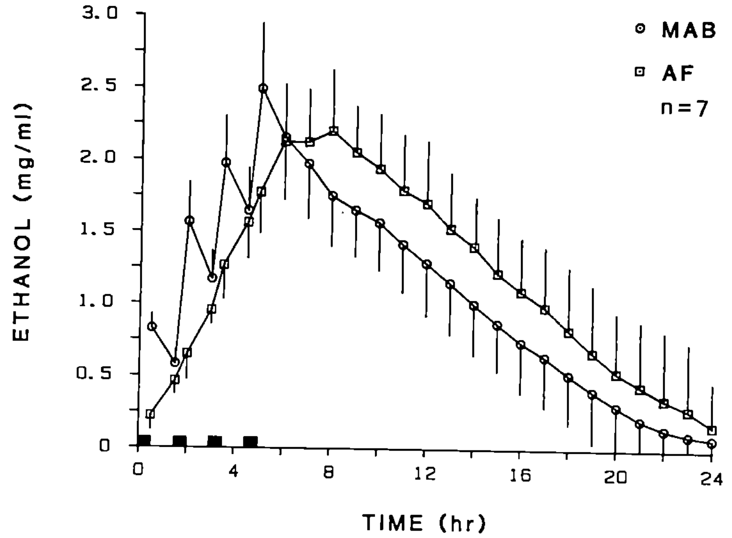

Fig. 3. Ethanol concentration–time curves for maternal arterial blood *(MAB)* and amniotic fluid *(AF)* of the near-term pregnant ewe (n = 7) during and after maternal administration of ethanol. Other denotations are described in the legend to Fig. 1.

arterial blood ethanol concentration–time curve. The maximum velocity was 0.141 ± 0.020 mg · ml^{-1} · hr^{-1}, and the Michaelis constant for ethanol was 0.056 ± 0.044 mg/ml (1.23 ± 0.96 mmol/L), which is similar to the Michaelis constant of 1 to 2 mmol/L ethanol for adult mammalian liver alcohol dehydrogenase.[14]

The mean ethanol concentration–time curves for fetal arterial blood and maternal arterial blood were virtually identical throughout the 24-hour study (Fig. 2), and the maximal fetal arterial blood ethanol concentration (2.524 ± 0.439 mg/ml) occurred at 5 hours. These data were indicative of rapid bidirectional placental transfer of ethanol between the mother and the fetus.

The mean ethanol concentration–time curves for amniotic fluid and maternal arterial blood are presented in Fig. 3. Transfer of ethanol into amniotic fluid was delayed, as reflected by the significantly lower ethanol concentration in amniotic fluid compared with maternal arterial blood and fetal arterial blood (Fig. 2) ($p < 0.05$) during the 5-hour period of maternal ethanol administration. The maximal amniotic fluid eth-

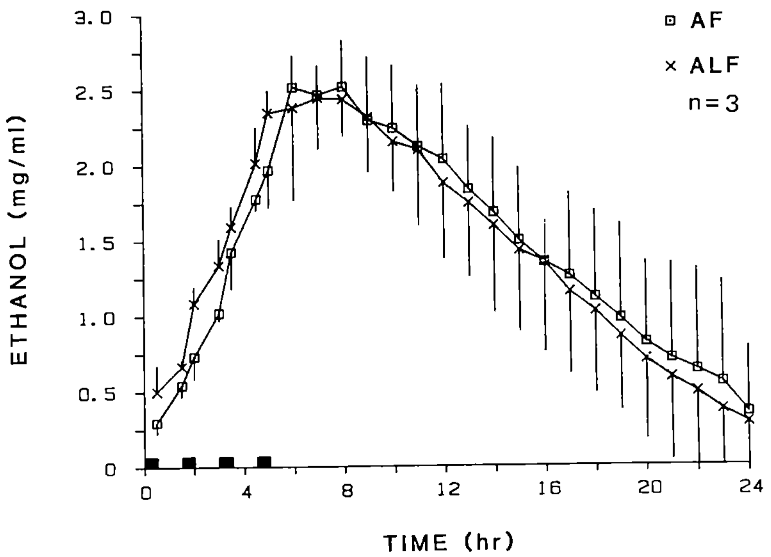

Fig. 4. Ethanol concentration–time curves for amniotic fluid *(AF)* and allantoic fluid *(ALF)* of the near-term pregnant ewe (n = 3) during and after maternal administration of ethanol. Other denotations are described in the legend to Fig. 1.

anol concentration was 2.185 ± 0.389 mg/ml, and the time of occurrence ranged from 6 to 9 hours for the individual animals. The maximal amniotic fluid ethanol concentration was significantly less ($p < 0.05$) than the peak ethanol concentration in each of maternal arterial blood and fetal arterial blood at 5 hours. There was a slow decline in amniotic fluid ethanol concentration during the 10- to 24-hour interval, consisting of an apparent linear phase and a terminal curvilinear phase. Throughout the elimination phase the amniotic fluid ethanol concentration was significantly greater ($p < 0.05$) than the maternal arterial blood and fetal arterial blood ethanol concentrations.

The mean ethanol concentration–time curves for allantoic fluid and amniotic fluid of three pregnant ewes are presented in Fig. 4. The allantoic fluid and amniotic fluid ethanol concentration–time curves were similar throughout the 24-hour study, which indicated that there was free exchange of ethanol between the two fluid sacs. The maximal ethanol concentration in allantoic fluid was 2.693 ± 0.301 mg/ml and occurred during the 5- to 9-hour interval for the individual animals. The maximal amniotic fluid ethanol concentration was 2.554 ± 0.212 mg/ml, and the time of occurrence ranged from 6 to 8 hours. For these three ewes the maximal ethanol concentration in maternal arterial blood and fetal arterial blood was 2.873 ± 0.365 mg/ml and 2.846 ± 0.462 mg/ml, respectively, and occurred at 5 hours.

The ethanol concentration–time curves for maternal arterial blood, fetal arterial blood, amniotic fluid, and allantoic fluid indicated that the ethanol concentrations in these biologic fluids decreased in parallel during the elimination phase (Figs. 2 to 4). The mean ethanol elimination rate values for maternal arterial blood, fetal arterial blood, and amniotic fluid of the seven pregnant ewes were 0.133 ± 0.017 $mg \cdot ml^{-1} \cdot hr^{-1}$, 0.134 ± 0.016 $mg \cdot ml^{-1} \cdot hr^{-1}$, and 0.145 ± 0.021 $mg \cdot ml^{-1} \cdot hr^{-1}$, respectively, and these data were not statistically different. The mean ethanol elimination rate for allantoic fluid, determined in three ewes, was 0.153 ± 0.017 $mg \cdot ml^{-1} \cdot hr^{-1}$, which was similar to the amniotic fluid ethanol elimination rate of 0.153 ± 0.027 $mg \cdot ml^{-1} \cdot hr^{-1}$ for these animals. There was no significant difference between the maximum velocity (0.141 ± 0.020 $mg \cdot ml^{-1} \cdot hr^{-1}$) and the ethanol elimination rate (0.133 ± 0.017 $mg \cdot ml^{-1} \cdot hr^{-1}$), determined from the maternal arterial blood ethanol concentration data of the seven ewes.

Ethanol-derived acetaldehyde was measurable in maternal arterial blood, fetal arterial blood, amniotic fluid, and allantoic fluid. The mean acetaldehyde concentration–time curves for maternal arterial blood, fetal arterial blood, and amniotic fluid of four pregnant ewes are presented in Fig. 5. The acetaldehyde concentrations were about a thousandfold less than the respective ethanol concentrations (Figs. 2 and 3) and were variable among animals, as indicated by the SD values. For each biologic fluid the acetaldehyde and ethanol concentration–time curves were not parallel. The maternal arterial blood acetaldehyde concentration was significantly greater ($p < 0.05$) than each of

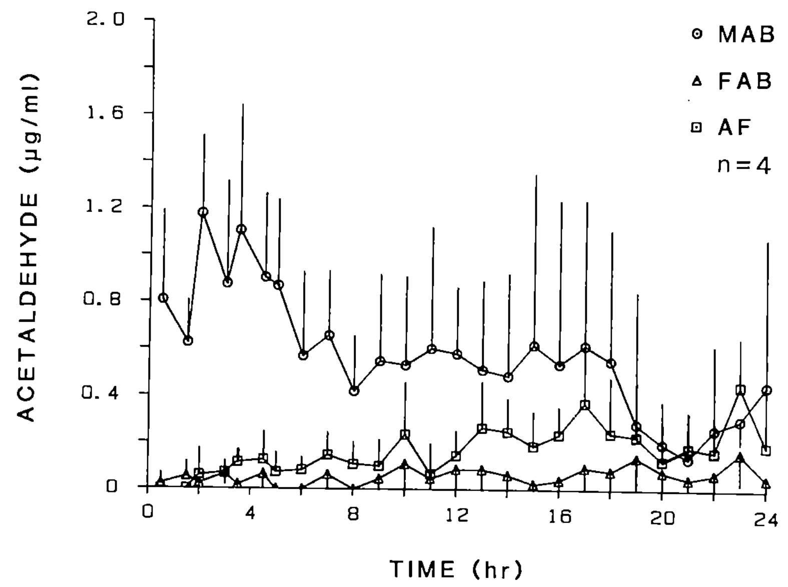

Fig. 5. Acetaldehyde concentration–time curves for maternal arterial blood *(MAB)*, fetal arterial blood *(FAB)*, and amniotic fluid *(AF)* of the near-term pregnant ewe (n = 4) during and after maternal administration of ethanol. Other denotations are described in the legend to Fig. 1.

the fetal arterial blood and amniotic fluid acetaldehyde concentrations during the 0.5- to 13-hour interval of the study.

Comment

The pharmacokinetics of acute, multiple-dose ethanol in the near-term pregnant ewe can be described by a four-compartment open model as shown in Fig. 6, consisting of maternal, fetal, amniotic fluid, and allantoic fluid compartments.* The data of the present study have established the unimpeded bidirectional placental transfer of ethanol between the maternal and fetal compartments and the free exchange of ethanol between the amniotic fluid and allantoic fluid compartments via diffusion across the amniotic and allantoic membranes. It is postulated that ethanol is distributed into the allantoic fluid and amniotic fluid via diffusion across the decidua and fetal membranes (chorion, allantois, and amnion) from the maternal circulation and/or across the fetal membranes from the fetal circulation. Fetal urinary excretion of ethanol also may occur into the amniotic fluid via the urethra and into the allantoic fluid via the urachus in view of the fact that in the near-term pregnant ewe, the major source of fluid in the amniotic and allantoic sacs is fetal urine.[11] Elimination of ethanol from the allantoic fluid and amniotic fluid may occur via diffusion across membranes into the fetal and/or maternal circulation and by fetal swallowing of amniotic fluid and subsequent absorption of ethanol from the gastrointestinal tract into the fetal circulation. The amniotic fluid and allantoic fluid may serve as a reservoir for ethanol in utero as indicated by the higher ethanol concentrations in these fluids compared with maternal arterial blood and fetal arterial blood during the elimination phase. The similar Michaelis constant (ethanol) values for in vivo biotransformation of ethanol in the maternal compartment and for adult mammalian liver alcohol dehydrogenase[14] and the superimposing maternal arterial blood and fetal arterial blood ethanol concentration–time curves indicate that alcohol dehydrogenase–catalyzed biotransformation of ethanol in the ewe is primarily involved in regulating the elimination of ethanol from the maternal-fetal unit. The similar ethanol elimination rate data for maternal arterial blood, fetal arterial blood, amniotic fluid, and allantoic fluid indicate that maternal hepatic biotransformation of ethanol ultimately regulates the elimination of ethanol from the amniotic fluid and allantoic fluid.

The rapid unimpeded bidirectional placental transfer of ethanol between the maternal and fetal compartments observed in this study is at variance with the data of an investigation in the anesthetized pregnant monkey, which demonstrated impaired placental transfer of ethanol resulting in fetal umbilical venous blood ethanol concentration that was about one third of the maternal uterine arterial blood ethanol concentration

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*One of the anatomic differences between the fetal lamb and the human fetus involves the intrauterine environment. Both the human fetus and the ovine fetus are located within the amniotic sac. In the case of the fetal lamb, however, there also is the allantoic sac that surrounds the amniotic sac.

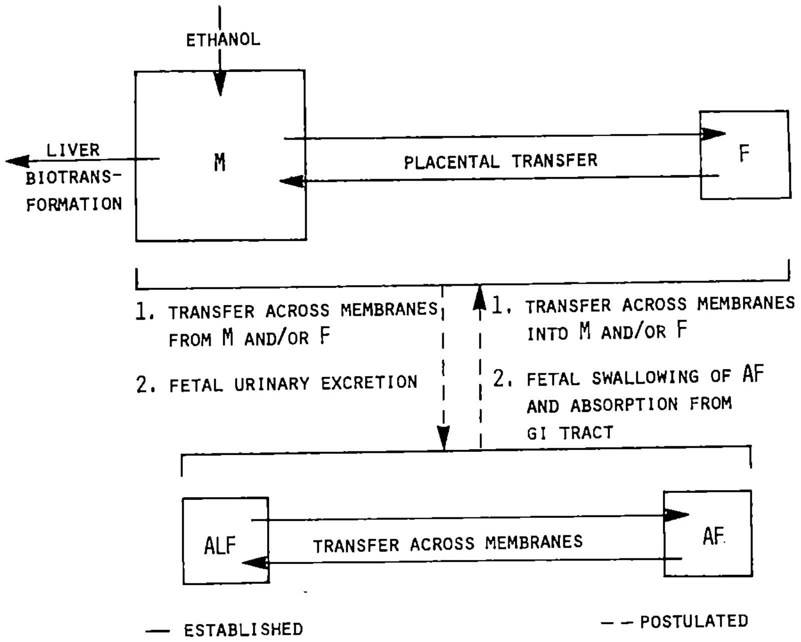
ETHANOL
LIVER BIOTRANSFORMATION
M
PLACENTAL TRANSFER
F
1. TRANSFER ACROSS MEMBRANES FROM M AND/OR F
2. FETAL URINARY EXCRETION
1. TRANSFER ACROSS MEMBRANES INTO M AND/OR F
2. FETAL SWALLOWING OF AF AND ABSORPTION FROM GI TRACT
ALF
TRANSFER ACROSS MEMBRANES
AF
— ESTABLISHED
– – POSTULATED

Fig. 6. Four-compartment open model of the pharmacokinetics of ethanol in the mother *(M)*, fetus *(F)*, amniotic fluid *(AF)* and allantoic fluid *(ALF)* of the near-term pregnant ewe. Established (——) and postulated (– –) pharmacokinetic processes are presented in the model.

during the 1.5-hour study.[8] In the latter study, 3 gm ethanol/kg maternal body weight was administered by maternal intravenous bolus injection over 1 to 2 minutes, which resulted in transient collapse of the umbilical vasculature and fetal hypoxia and acidosis. These results must be interpreted with caution, since the ethanol dosage regimen does not mimic any human alcohol drinking pattern and would have resulted in toxic maternal blood ethanol concentration immediately after bolus injection. It is noteworthy that the results of the present study are consistent with the pharmacokinetic data in the near-term pregnant guinea pig that received oral administration of four dosages of 1 gm ethanol/kg maternal body weight over 3 hours, in which the maternal blood and fetal blood ethanol concentrations were similar during the 26-hour study.[15]

The proximate ethanol metabolite, acetaldehyde, was measurable in maternal arterial blood, fetal arterial blood, amniotic fluid, and allantoic fluid at concentrations that were at least a thousandfold lower than the respective ethanol concentrations. In maternal arterial blood the lower acetaldehyde concentration compared with the ethanol concentration indicates that only a small amount of acetaldehyde is not oxidized by maternal hepatic aldehyde dehydrogenase, the enzyme primarily involved in the biotransformation of acetaldehyde to acetate.[14] It would appear that the maternal hepatic aldehyde dehydrogenase activity is sufficient to oxidize most of the ethanol-derived acetaldehyde over a wide ethanol concentration range, as the maternal arterial blood acetaldehyde and ethanol concentration–time curves were not parallel. The presence of acetaldehyde in fetal arterial blood may be due primarily to transfer of this ethanol metabolite from the maternal circulation, as the fetal liver had <10% of the alcohol dehydrogenase activity that was present in the maternal liver for the oxidation of ethanol to acetaldehyde.[16] The lower acetaldehyde concentration in fetal arterial blood compared with maternal arterial blood indicates that there is acetaldehyde-oxidizing capacity in the maternal-fetal unit in addition to that in the maternal liver, which could regulate the amount of this metabolite distributed from the mother into the fetal systemic circulation. In this regard, it has been observed in the near-term pregnant guinea pig that aldehyde dehydrogenase was present in the fetal liver with activity similar to that in the maternal liver.[17] If this is also the case for the near-term pregnant ewe, most of the acetaldehyde transferred across the placenta could be oxidized by fetal liver aldehyde dehydrogenase.

In summary, the data of this study indicate that for maternal administration of acute, multiple-dose ethanol during near-term pregnancy that would mimic a binge-type drinking episode, there is unimpeded bidirectional placental transfer of ethanol between the mother and the fetus; the amniotic fluid surrounding the fetus is a reservoir for ethanol in utero; elimination of ethanol from the maternal-fetal unit including the amniotic fluid is regulated by maternal hepatic biotransformation of ethanol catalyzed by alcohol dehydrogenase; and there is appreciable acetaldehyde-oxidizing capacity in the maternal liver and at extrahepatic sites. We have demonstrated in our laboratory that the pharmacokinetics of ethanol and its proximate metabolite, acetaldehyde, during near-term pregnancy are similar for administration to the mother of acute low-dose[7] and high-dose ethanol regimens. The effect of repeated, daily administration of ethanol on the pharmacokinetics of ethanol and acetaldehyde in the maternal-fetal unit remains to be determined.

REFERENCES

1. Rosett HL, Weiner L. Alcohol and pregnancy: a clinical perspective. Annu Rev Med 1985;36:73.
2. Abel EL. Consumption of alcohol during pregnancy: a review of effects on growth and development of offspring. Hum Biol 1982;54:421.
3. McLeod W, Brien J, Loomis C, Carmichael L, Probert C, Patrick J. Effect of maternal ethanol ingestion on fetal breathing movements, gross body movements, and heart rate at 37 to 40 weeks' gestational age. AM J OBSTET GYNECOL 1983;145:251.
4. Ng PK, Cottle MK, Baker JM, Johnson B, Van Muyden P, Van Petten GR. Ethanol kinetics during pregnancy. Study in ewes and their fetuses. Prog Neuropsychopharmacol Biol Psychiatry 1982;6:37.
5. Hill DE, Slikker W Jr, Goad PT, Bailey JR, Sziszak TJ, Hendrickx AG. Maternal, fetal, and neonatal elimination of ethanol in nonhuman primates. Dev Pharmacol Ther 1983;6:259.
6. Cumming ME, Ong BY, Wade JG, Sitar DS. Maternal and fetal ethanol pharmacokinetics and cardiovascular responses in near-term pregnant sheep. Can J Physiol Pharmacol 1984;62:1435.
7. Brien JF, Clarke DW, Richardson B, Patrick J. Disposition of ethanol in maternal blood, fetal blood, and amniotic fluid of third-trimester pregnant ewes. AM J OBSTET GYNECOL 1985;152:583.
8. Mukherjee AB, Hodgen GD. Maternal ethanol exposure induces transient impairment of umbilical circulation and fetal hypoxia in monkeys. Science 1982;218:700.
9. O'Shea KS, Kaufman MH. The teratogenic effect of acetaldehyde: implications for the study of the fetal alcohol syndrome. J Anat 1979;128:65.
10. Blakley PM, Scott WJ Jr. Determination of the proximate teratogen of the mouse fetal alcohol syndrome. 1. Teratogenicity of ethanol and acetaldehyde. Toxicol Appl Pharmacol 1984;72:355.
11. Szeto HH. Pharmacokinetics in the ovine maternal-fetal unit. Annu Rev Pharmacol Toxicol 1982;22:221.
12. Steenaart NAE, Clarke DW, Brien JF. Gas-liquid chromatographic analysis of ethanol and acetaldehyde in blood with minimal artifactual acetaldehyde formation. J Pharmacol Methods 1985;14:199.
13. Wilkinson PK. Pharmacokinetics of ethanol: a review. Alcoholism (NY) 1980;4:6.
14. Khanna JM, Israel Y. Ethanol metabolism. Int Rev Physiol 1980;21:275.
15. Clarke DW, Steenaart NAE, Slack CJ, Brien JF. Pharmacokinetics of ethanol and its metabolite, acetaldehyde, and fetolethality in the third-trimester pregnant guinea pig for oral administration of acute, multiple-dose ethanol. Can J Physiol Pharmacol 1986;64:1060.
16. Cumming ME, Ong BY, Wade JG, Sitar DS. Ethanol disposition in newborn lambs and comparison of alcohol dehydrogenase activity in placenta and maternal sheep, fetal and neonatal lamb liver. Dev Pharmacol Ther 1985;8:338.
17. Clarke DW, Steenaart NAE, Brien JF. Disposition of ethanol and activity of hepatic and placental alcohol dehydrogenase and aldehyde dehydrogenases in the third-trimester pregnant guinea pig for single and short-term oral ethanol administration. Alcoholism (NY) 1986;10:330.

CORRESPONDENCE

Paper speed in electronic fetal heart rate monitoring

To the Editors:

I feel that comment is necessary regarding a letter by Dr. Robert C. Goodlin, entitled "Electronic fetal monitoring as seen through the looking glass" (AM J OBSTET GYNECOL 1987;155:454-5). He describes the fetal heart rate recording in his Fig. 1 as "'not worrisome enough for a scalp pH.'" At first glance, I would agree, but what disturbs me is that, on closer observation, this tracing is obtained at a speed of 2 rather than 3 cm/min. I believe that if this tracing were obtained at a speed of 3 cm/min a sinusoidal pattern with little or no short-term variability would be seen. There was no response to scalp stimulation. All of this would be an ominous pattern, especially if there was no response to intrauterine resuscitative efforts. It would be interesting to see more of the preceding strip.

I am under the impression that 3 cm/min is the "standard" speed to use in electronic monitoring as the slower speeds tend to give artificial short-term variability and also to mask other subtle changes, for instance, subtle late decelerations.

I practice in a small, rural level 1 hospital with no capability for scalp pH determination and I rely exclusively on pattern interpretation with special emphasis on the presence or absence of short-term variability and on fetal response to scalp stimulation if the occasion arises.

I would like to hear from others regarding this particular tracing and also would like their comments on the speed of the tracings obtained with electronic fetal monitoring.

John S. Floyd III, M.D.

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2306 Harrington Street
Newberry, South Carolina 29108

Reply

To the Editors:

Dr. Floyd's letter reinforced one of my impressions about fetal heart rate tracings, to wit, that given an asphyxiated newborn infant, someone will declare its fetal heart rate tracing abnormal, regardless of the pattern. I would agree with most any interpretation of this tracing except that it was sinusoidal.

For years I proposed a monitor speed of 1 cm/min (as used in Europe) as a measure to reduce paper cost and storage problems. I have worked with paper speeds varying from 0.5 to 50 cm/min and believe that they can all be used successfully. In my opinion, there is no magic in any particular paper speed.

As noted in the legend to Fig. 1 of my letter, the fetal heart rate recording was that immediately *before* the scalp sample was obtained. While the recording was less than perfect (it was noisy), there did appear to be an acceleration with or immediately after the first scalp sample. The subsequent scalp samples were not associated with a reassuring fetal heart rate pattern.

I am skeptical that the fetal stimulation technique will solve Dr. Floyd's problems. In my experience, 60% of fetuses with persistent late decelerations will have normal scalp pH values and 70% of fetuses not responding to scalp stimulation will have no significant acidemia. In addition to the present case, I have reviewed other records of fetal acidemia in which the fetus likewise appeared initially to respond to scalp stimulation with fetal heart rate accelerations.

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Screening for genetic anomalies

To the Editors:

A small correction is needed in Dr. James N. Macri's mathematics in his article, "Critical issues in prenatal maternal serum α-fetoprotein screening for genetic anomalies" (AM J OBSTET GYNECOL 1986;155:240-6): $1/295 \approx 0.003 \approx 0.3\%$, not 0.003%.

Newell R. Washburn, M.D.

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Reply

To the Editors:

I am thankful to Dr. Washburn for correcting the mathematical/typographic error in my article, "Critical issues in prenatal maternal serum α-fetoprotein screening for genetic anomalies." I agree that it should have read 0.3% instead of 0.003%.

The point made by Dr. Washburn relates to the rate of occurrence of neural tube defects in pregnancies in older women, which, in a broader context, relates to: (1) effective communication of maternal serum α-fetoprotein screening results to patients and (2) informed procreative decision making.

The relevance of the correction is noteworthy. In order to provide accurate and scientifically valid results in maternal serum α-fetoprotein screening, reports to physicians must be based on far more than α-fetoprotein quantitation; for example: (1) incidence patterns vary among a number of subgroups of gravid women; (2) incidence patterns and all other relevant clinical factors as well as α-fetoprotein quantitative levels should be taken into account in computing and reporting prior risks and patient-specific posterior risks; (3) the use of multiples of the median fails to provide physicians and patients with information that is clinically relevant for making informed decisions on procreation.

James N. Macri, Ph.D.

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Criteria for interpretation of a reactive nonstress test

To the Editors:

The article by Erskine et al., entitled "Failure of nonstress test and Doppler-assessed umbilical arterial blood flow to detect imminent intrauterine death" (Am J Obstet Gynecol 1986;154:1109-10), described a case of intrauterine fetal death 12 hours after a reactive nonstress test. The criteria for a reactive nonstress test vary among institutions; therefore I was pleased to see a portion of the actual fetal heart rate tracing included in the article. If one uses the generally accepted criteria of two accelerations of 15 bpm and of 15 seconds' duration,[1] I cannot interpret the monitor strip shown as reactive. The acceleration associated with the first fetal movement does meet the above criteria, but the three subsequent accelerations are of inadequate duration. It would be beneficial for the authors to define their criteria for a reactive nonstress test and demonstrate that the criteria were in fact met.

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REFERENCE

1. Patkos P, Boucher M, Broussard PM, Phelan JP, Platt LD. Factors influencing nonstress test results in multiple gestations. Am J Obstet Gynecol 1986;154:1107-8.

Reply

To the Editors:

The nonstress test reported in our article was reactive by the standard criteria. The section illustrated was intended to be an example of the total recording.

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J. W. K. Ritchie, M.D.
A. Zaltz, B.Sc., M.D.
T. Tice, R.N.

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Risk of vaginal delivery after a prior cesarean section

To the Editors:

Finley and Gibbs are to be applauded for their fine recent article regarding the (in)frequency of need for emergent interventions in women undergoing a trial of labor (Am J Obstet Gynecol 1986;155:936-9). Among 1156 cases, rapid start-up time for operation was critical in only one patient. The authors questioned whether patients with a single prior low transverse cesarean section are at increased risk for intrapartum emergencies.

In a limited experience I have encountered two cases in which an in-house operating team saved the lives of women who had vaginal deliveries after single, prior low transverse cesarean sections. Profuse vaginal bleeding and shock followed delivery with each patient. Estimated blood loss within 10 minutes of delivery was >3000 ml in each patient, despite firm uterine contraction; emergency hysterectomy was begun within 20 minutes of delivery. Placenta accreta was the clinical diagnosis in one case but was not proven histologically. Placenta increta was present in the other patient.

In women of low parity, postpartum hysterectomy is rarely required; the most common indications are placenta accreta, percreta, increta, and hemorrhage. Abnormally adherent placentas are increased in frequency with even a single prior cesarean section, and it may well be that the dominant risk of trial of labor after prior low transverse cesarean does not involve uterine scar dehiscence but placenta accreta, increta, percreta, and maternal exsangiunation from postpartum bleeding.

The excellent safety record for trials of labor after cesarean section has been established in institutions with rapid response times for emergency intervention. Should vaginal delivery after cesarean section be considered elsewhere?

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Langerhans' cells in the cervix

To the Editors:

We were interested to read the article entitled "Quantitative assessment of Langerhans' cells in cervical intraepithelial neoplasia and wart virus infection" (Am J Obstet Gynecol 1986;154:509-15), but we question some of their comments.

Our own interest in Langerhans' cells followed their observation during reestablishment of cervical epithelium after laser treatment of intra-epithelial neoplasia.[1] Initially we used adenosine triphosphatase, but more recently we are using a variety of techniques, including monoclonal antibodies (NA1/34 and major histocompatibility complex class II antigens), zinc, iodide, and osmium, with electron microscopy in addition to S-100. Different techniques show a variation in Langerhans' cell numbers and distribution. Class II antigens have been shown to mark 60% of those Langerhans' cells positive for NA1/34 (T-6 antigen), whereas S-100 only marked 35%.[2] In addition McArdle and Muller have shown up to a 100-fold range in S-100 cells in normal and abnormal cervices. Thus S-100 is a poor marker for Langerhans' cells, and the authors make no mention of other S-100–positive cells in squamous epithelium.[3] We also wonder if the authors have any data to dismiss the possibility of loss of S-100 positivity with storage of blocks up to 6 years.

The reduction in Langerhans' cells shown by McArdle and Muller and by Morris et al.[4] in association with wart virus infection may represent increased Langerhans' cells recirculation to lymph nodes or to a cytopathic effect. Alternatively, their findings may represent loss of antigen expression or markers from the Langerhans' cells. Persistence of cells containing Birbeck granules but not expressing other antigens would be analogous to the situation following exposure of skin to ultraviolet light.[5] We are thus using various techniques to investigate whether the reduction in Langerhans' cells in the cervix with human papillomavirus infection are real or apparent.

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REFERENCES

1. MacLean AB. Cervical healing and Langerhans' cells. Br J Obstet Gynaecol 1984;91:1145-8.
2. Tay SK, Jenkins D, Maddox P, Campion M, Singer A. Subpopulations of Langerhans' cells in cervical neoplasia. Br J Obstet Gynaecol 1987;94:10-5.
3. Takahashi K, Isobe T, Ohtsuki Y, Sonobe H, Yamaguchi H, Akagi T. S-100 protein positive human T-lymphocyte. Am J Clin Pathol 1985;83:69-72.
4. Morris HB, Gatter KC, Sykes G, Casemore V, Mason DY. Langerhans' cells in human cervical epithelium: effects of wart virus infection and intra-epithelial neoplasia. Br J Obstet Gynaecol 1983;90:412-20.
5. Aberer W, Schuler G, Stingl G, Honigsmann H, Wolff K. Ultraviolet light depletes surface markers of Langerhans' cells. J Invest Dermatol 1981;76:202-12.

Reply

To the Editors:

Drs. Hawthorn and MacLean raise several questions with regard to our article on Langerhans' cells in the cervix. We agree with their observation that different techniques show a variation in Langerhans' cell numbers and distribution. In our own laboratory we have used adenosine triphosphatase, monoclonal antibodies to OKT_6 and HLA-DR, and electron microscopy to study Langerhans' cells in skin, and we agree roughly with the percentages they quote. However, this discrepancy is already well documented in the existing literature.[1]

We chose to use antibodies to S-100 antigen for two reasons. First, S-100 antigen immunoperoxidase can be used in paraffin sections, allowing greater histologic accuracy when assessing varying grades of cervical intraepithelial neoplasia. Second, as Drs. Hawthorn and MacLean point out in their last paragraph, significant numbers of epidermal Langerhans' cells persist after ultraviolet β-radiation, even though expression of adenosine triphosphatase and HLA-DR is virtually eliminated.[2] Because S-100 protein antigen is diffusely present in the Langerhans' cells cytoplasm and nucleus, we believe that it would not be as susceptible to modulation as cell membrane–associated antigens, and although it marks fewer Langerhans' cells than other markers, it would be more accurate in assessing true changes in Langerhans' cell numbers in cervical epithelium. Although more recent evidence suggests that epithelial injury may result in increased S-100 antigen expression,[1] there is still no evidence for decreased expression as is found with the membrane-associated antigens.

Increased Langerhans' cells density in cervical intraepithelial neoplasia has now been shown by three separate markers NAI/34,[3] S-100 protein,[4] and a zinciodide-osmium procedure[5] and thus is likely to reflect a true increase in intraepithelial Langerhans' cells. Similarly, Langerhans' cell depletion in wart virus infection has been shown with both NAI/34[3] and S-100 protein.[4]

Although there is a problem with the use of antibody to S-100 antigen to assess Langerhans' cell numbers in skin because of the presence of basal melanocytes (we have published a double-staining procedure[6] to overcome this problem), the same problem does not exist in cervical epithelium. Finally, although the vast majority of the biopsies studied were done recently, we have found preservation of S-100 protein antigenicity in paraffin blocks stored up to 16 years in our laboratory.

We would like to thank Drs. Hawthorn and MacLean for their comments and look forward to the publication of their results.

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REFERENCES

1. Schneider SA, Fukuyama K, Maceira J, Epstein WL. Effect of Ultraviolet β radiation on S-100 protein antigen in epidermal Langerhans' cells. J Invest Dermatol 1985;84:146-8.
2. Aberer W, Schuler G, Stingl G, Honigsmann H; Wolff K. Ultraviolet light depletes surface markers of Langerhans' cells. J Invest Dermatol 1981;76:202-10.
3. Morris HB, Gatter KC, Sykes G, Casemore V, Mason DY. Langerhans' cells in human cervical epithelium: effects of

wart virus infection and intra-epithelial neoplasia. Br J Obstet Gynaecol 1983;90:412-20.
4. McArdle JP, Muller HK. Quantitative assessment of Langerhans' cells in cervical intra-epithelial neoplasia and wart virus infection. AM J OBSTET GYNECOL 1986;159:509-15.
5. Caorsi I, Figueroa CD. Langerhans' cells in squamous exocervical carinoma: a quantitative and ultrastructural study. Ultrastruct Pathol 1984;7:25-40.
6. Halliday GM, McArdle JP, Knight BA, Muller HK. New methodology for assessment of the Langerhans' cell network. J Pathol 1986;148:127-34.

Morality of induced abortion and freedom of choice

To the Editors:

It was a pleasure to read the presidential address, "Your morality or mine? An inquiry into the ethics of human reproduction," by Hill (AM J OBSTET GYNECOL 1986;154:1173-80), but I question the discussion of sex selection, "of intentionally aborting a fetus of an unwanted sex," being wrong because such a decision is equated with sexism.

If a fetus may be aborted without a reason being required, why should a considered decision and action arrived at with reason be wrong? It may well be that an abortion is being requested because the year in which the pregnancy occurs a house is being bought or an expensive vacation has been planned, and the pregnancy and the house and/or vacation cannot both be afforded, or the pregnancy would in some other nonfinancial manner interfere with another choice.

Therefore, if induced abortion may be obtained without having to provide any reason, why should a reason that is believed to be important by the person or persons desiring the abortion be considered wrong? Who is imposing moral standards on whom? Whose morality is more valid and less wrong—the aborter's or the abortee's?

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This is a personal communication and not to be construed in any way as being representative of Kaiser Permanente/Southern California Permanente Medical Group.

Reply

To the Editors:

I appreciate Dr. Fribourg's interest in my review of the current literature covering the ethics of human reproduction, and I can understand his query.

In the address I was careful to point out that there is still considerable doubt in the minds of many of the morality of "abortion on demand." I firmly believe that abortion can be morally justified if it is done to ease a significant burden to the patient or to her family or society. In my mind there is still something immoral about an abortion because a pregnancy is "inconvenient," and I would certainly place a higher priority on a pregnancy than on "an expensive vacation." However, as Dr. Fribourg points out, that is my morality, and I would not impose it on a patient. Nevertheless, I do believe that it is the physician's responsibility to discuss all of the implications of interrupting a pregnancy with the patient, taking into account her desires, beliefs, aims, and goals. As I stated in my presentation, the physician must help the patient to make a decision that can be lived with, and if that decision is for abortion, then it should be done as early and as safely as possible.

As to the issue of sexism, I wished merely to point out that if one accepts the premise that sexual discrimination is wrong, then it naturally follows that abortion of a fetus because it is an unwanted sex is also wrong.

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Increased boy-to-girl ratio in women with preterm labor beginning with contractions

To the Editors:

The letter by MacGillivray and Davey, entitled "The influence of fetal sex on rupture of the membranes and preterm labor" (AM J OBSTET GYNECOL 1985;153:814), demonstrated that the overall boy-to-girl ratio at delivery before 37 weeks of gestation was higher than that found in the total Cape Colored population (Cape Town, South Africa). Furthermore, they observed a dramatic preponderance of boys over girls in women with spontaneous rupture of the membranes, but not in women with preterm labor that begins with contractions as compared with the sex ratio in the total population. They interpreted this finding as an indication that the amniotic fluid and membranes are more likely to become infected in pregnancies with a male fetus.

To confirm the results of MacGillivray and Davey, we studied the records of Japanese women admitted to the National Defense Medical College Hospital in 1978 to 1985. Before 37 weeks of gestation, 216 women were delivered of infants, and these women were divided into those with boy babies and those with girl babies.

Table I. Spontaneous preterm labor in Japanese women (singleton births) from National Defense Medical College Hospital records for 1978 to 1985

Gestation (wk)	*No. of boys*	*No. of girls*	*All (n)*	*Boy-to-girl ratio*
24-27	6	3	9	2.00:1
28-30	8	7	15	1.14:1
31-33	15	14	29	1.07:1
34-36	102	61	163	1.67:1
Total	131	85	216	1.54:1

Table II. Onset of labor

Gestation (wk)	Contractions			Spontaneous rupture of membranes		
	No. of boys	No. of girls	Boy-to-girl ratio	No. of boys	No. of girls	Boy-to-girl ratio
24-27	1	3		5	0	
28-30	6	3		2	4	
31-33	9	7		6	7	
34-36	55	29		47	32	
Total	71	42	1.69 : 1	60	43	1.40 : 1

The distribution of births by sex and the overall ratios of boy deliveries to girl deliveries by gestational age are shown in Table I. The cases were further divided into those with the onset of preterm labor with contractions and those with the onset of labor with spontaneous rupture of the membranes (Table II). In agreement with the results of MacGillivray and Davey, the overall boy-to-girl ratio at delivery before 37 weeks of gestation was 1.54 : 1, which is higher than the 1.02 : 1 ratio found in the total population of the city of Tokorozawa, where our hospital is located. We were able to confirm the association of boy babies with spontaneous rupture of the membranes (Table II). The finding appears to support the postulate of MacGillivray and Davey that the amniotic fluid and membranes are more likely to become infected in pregnancies with a male fetus.

In contrast to the results of MacGillivray and Davey, the boy-to-girl ratio was also increased in women with preterm labor that began with contractions. The boy-to-girl ratio was not significantly different in women with spontaneous rupture of the membranes and those with preterm labor that began with contractions (χ^2 test). The reason for the discrepancy between the results of MacGillivray and Davey and ours is unknown. The racial difference in the populations studied may account for it.

In conclusion, the boy-to-girl ratio was increased in women with preterm labor that began with contractions and in those with spontaneous rupture of the membranes. Therefore factors other than rupture of the membranes should also be considered as possible causes of the increased boy-to-girl ratio in women with preterm labor.

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Amniotic fluid discoloration caused by blood

To the Editors:

We are delighted to see another study confirming our finding that blood is usually the pigment that accounts for the discoloration of amniotic fluid noted occasionally at midtrimester amniocentesis (Zorn EM, Hanson FW, Greve LC, Phelps-Sandall B, Tennant FR. Analysis of the significance of discolored amniotic fluid detected at midtrimester amniocentesis. Am J Obstet Gynecol 1986;154:1234-40). However, we wish to make a correction. In their introduction, Zorn et al. state, "Surprisingly, little investigation has been done into the cause of the discoloration. Various authors have assumed it to be blood . . . ," at which point they reference our article.[1] Actually we did investigate the cause of the discoloration, and we did not assume the pigment to be blood; we demonstrated that the discoloration was caused by heme pigments. We believe this is an important semantic difference. However, the study by Zorn et al. provides an important adjunct to our data through their use of sodium dodecyl sulfate–polyacrylamide gel electrophoresis of the discolored amniotic fluid. We do not quibble with their demonstration that in at least a small number of samples electrophoresis revealed the presence of fetal gut contents rather than hemoglobin. We also do not quibble with their conclusion and the conclusion of Allen[2] that the finding of discolored fluid at amniocentesis may portend a slightly increased risk of spontaneous abortion, although in our study we failed to demonstrate such a risk.

We agree also with the statements of Zorn et al. and of Allen (in his discussion of the article by Zorn et al.) that the presence of discolored amniotic fluid obtained at the time of midtrimester amniocentesis should be documented in the medical record and discussed (cautiously but optimistically) with the patient. We also recommend strongly that such fluids be tested with a Hemoccult card (SmithKline Diagnostics, Inc., Sunnyvale, California) and that the result of this test be recorded in the patient's medical record.

Finally, we hope that these recent excellent studies will put to rest the archaic view that the discoloration of midtrimester amniotic fluid obtained during amniocentesis is caused by meconium. The burning questions at the moment are whether the blood is of maternal or fetal origin and how such blood enters the amniotic fluid. We hope that Zorn et al., Allen, and others will continue to pursue these issues.

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2. Allen R. The significance of meconium in midtrimester genetic amniocentesis. AM J OBSTET GYNECOL 1985;152:413-7.

Reply

To the Editors:

In reading the letter by Strickland and Hankins, we see two areas where we disagree with their conclusions.

First, their contention that they demonstrated the presence of heme pigments in all of their monitored discolored fluids does not seem valid. The methods that they used for their determinations are subject to substantial error. The Hemoccult method is subject to false positives in approximately 12% of the cases tested.[1] The spectrophotometric methods used by Hankins et al.[2] may be diagnostic when applied to pure compounds, but when used in conjunction with fluids that may contain diverse molecular entities, the possibility of misinterpretation is high. Many nonheme compounds have an absorbance spectrum similar to heme, for example, uroporphyrin.[3] In fact, all tetrapyrroles where the nucleus is fully conjugated have absorbance maxima that could easily be concealed by the spectra displayed in the article by Hankins et al.

Second, on a more practical as opposed to theoretical basis, we[4] have shown that some discolored fluids, although an admittedly small proportion, contain additional pigments that are not hemoglobin. We have also shown that this pigment is electrophoretically identical to materials found in the fetal gut. This pigment undoubtedly contributed to the discoloration of the samples in which it was found.

Therefore, we believe that the use of the word *assumed* in our article, in relation to the findings of Hankins et al., was justified.

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2. Hankins GDV, Rowe J, Quirk JG, Trubey R, Strickland DM. Significance of brown and/or green amniotic fluid at the time of second trimester amniocentesis. Obstet Gynecol 1984;64:353-8.
3. Falk JE. Porphoryns and metalloporphoryns. New York: Elsevier Publishing, 1964.
4. Zorn EM, Hanson FW, Greve LC, Phelps-Sandall B, Tennant FR. Analysis and significance of discolored amniotic fluid detected at midtrimester amniocentesis. AM J OBSTET GYNECOL 1986;154:1234-40.

Correction

In the article by Shepard et al., entitled "Proportional weight gain and complications of pregnancy, labor, and delivery in healthy women of normal prepregnant stature" (AM J OBSTET GYNECOL 1986;155:947-54), on page 948, the third and fourth sentences of the fourth paragraph should have been: "The first multiple regression model was developed to identify factors associated with proportional weight gain and to estimate the proportion of weight gained, while adjusting for confounding variables. Next, multiple logistic regression models were developed to determine which complications of pregnancy, labor, and delivery were significantly associated with proportional weight gain, once again adjusting for confounding variables."

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McGowan, Kathleen, 333 N. First St., #150, Boise, ID 83702
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Montgomery, Curtis Edward; 6502 McAbee Road, San Jose, CA 95120
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Moyer, Kathryn L., 700 Arizona Ave., Santa Monica, CA 90401
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Norton, Kathleen Lynn, 207 Terrace Ave., San Rafael, CA 94901
Nystul, Stephen Robert, 1315 Ynex Pl., Coronado, CA 92118
O'Shea, John Gerard, 3212 Concord Dr., Orange, TX 77630
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Thompson, Rachel Adams, 7550 Fannin, Houston, TX 77054

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Woo, Kevin Scott, 6600 Bruceville Road, Sacramento, CA 95823
Wycheck, Jacqueline G., 4440 Magnolia St., Suite 400, New Orleans, LA 70115
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Yamaki, Estelle Ikuko, 4316 S.W. 307th St., Federal Way, WA 98023
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New certifications: Subspecialty of Maternal-Fetal Medicine

Date of examination: February 1987

Place: San Francisco, California

Number certified this examination: 54*

Cumulative total of certificates awarded for this subspecialty: 419

Abdella, Thomas Nelson, 853 Jefferson Ave., Memphis, TN 38163
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Barss, Vanessa Ajemian, 62 Richmond Road, Belmont, MA 02178
Block, Barry S., Dept. Ob/Gyn, Box 446, 5841 S. Maryland Ave., Chicago, IL 60637
Brown, Haywood L., 12930 Parlange Ct., New Orleans, LA 70128
Callan, Nancy Anne, Johns Hopkins Hosp., Dept. Ob/Gyn, Houck 228, Baltimore, MD 21205
Catalano, Patrick M., 7 Knoll Circle, S. Burlington, VT 05401
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Dix, Patricia Mary, Univ. Oklahoma Health Sci. Ctr., P. O. Box 26901, Dept. Ob/Gyn, Oklahoma City, OK 73190
Eden, Robert D., 414 Parkview Pl., Burr Ridge, IL 60521
Ernest, Joseph M., III, Bowman-Gray, Dept. Ob/Gyn, 300 S. Hawthorne Road, Winston-Salem, NC 27103
Evans, Arthur Thompson, III, 1837 Parliament Circle, Carmichael, CA 95608

The names of two newly certified diplomates are not included in this listing at their request.

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Ganesh, Vijaya Lakshmi, 5 Eccleston Ct., Montville, NJ 07045

Gimovsky, Martin L., 517 W. Orange Grove Ave., Sierra Madre, CA 91024

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Hankins, Gary D. V., 7601 Tippit Trail, San Antonio, TX 78240

Hariharan, Seemanthini, Michael Reese Hosp., Dept. Ob/Gyn, Lake Shore at 31st, Kap100, Chicago, IL 60616

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Kaufmann, Robert Charles, SIU School Med., 800 N. Rutledge, Dept. Ob/Gyn, Springfield, IL 62702

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Repke, John Thomas, 10 Dulaney Gate Ct., Cockeysville, MD 21030

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Spinnato, Joseph Anthony, II, 2451 Fillingim St., Mastin Room 512, Mobile, AL 36617

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Tropper, Pamela J., Columbia Presbyterian, 622 W. 168th St., Dept. Ob/Gyn, New York, NY 10032

Valenzuela, Guillermo J., Div Perinatal Biol., Loma Linda Univ. Med Ctr., Loma Linda, CA 92350

Veille, Jean-Claude Lucien, MacDonald Hosp. for Women, 2074 Abington Road, Cleveland, OH 44106

Wallace, Roger L., The Perinatal Center, 5275 F St., Sacramento, CA 95819

Warsof, Steven Lester, CHKD, Room 323, Div. Maternal-Fetal Med., 800 W. Olney Road, Norfolk, VA 23507

Watson, Dottie L., Hutzel Hosp., Dept. Ob/Gyn, 4707 St. Antoine, Detroit, MI 48201

Weinbaum, Paul J., 5 Cricket Lane, Worchester, MA 01602

Zuidema, Laura Joy, Univ. Illinois, Dept. Ob/Gyn, 840 S. Wood St., Chicago, IL 60612

New certifications: Subspecialty of Reproductive Endocrinology

Date of examination: February 1987

Place: San Francisco, California

Number certified this examination: 25

Cumulative total of certificates awarded for this subspecialty: 263

Blackwell, Richard E., Univ. Alabama, Dept. Ob/Gyn, 555 Old Hillman, Birmingham, AL 35294

Brenner, Steven Hal, Dept. Ob/Gyn, Long Island Jewish Med. Ctr., New Hyde Park, NY 11042

Collins, Robert Leon, Cleveland Clinic, Dept. Ob/Gyn, 9500 Euclid Ave., Cleveland, OH 44106

Damewood, Marian deMarinis, 5001 Springlake Way, Baltimore, MD 21212

De Leon, Frank D., #19 Brentwood Circle, Lubbock TX 79416

Dlugi, Alexander Michael, 66 Winchester St., Brookline, MA 02146

Eisenberg, Esther, Pennsylvania Hosp., Dept. Ob/Gyn, 1 Pine E., 8th & Spruce, Philadelphia, PA 19107

Fritz, Marc A., 611 Wilfred Ave., Dayton, OH 45410

Gianfortoni, Joseph Gene, 5805 Dorchester, #4C, Chicago, IL 60637

Hoffman, David I., Prentice 454, 333 E. Superior St., Chicago, IL 60611

Jones, Kirtly Parker, Dept. Ob/Gyn, Univ. Utah Med. Ctr., Salt Lake City, UT 84132

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Lewis, Vivian, University Hosp. Dept. Ob/Gyn, 840 S. Wood St., Chicago, IL 60612

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Lucas, John Albert, III, Univ. Med Ctr., Dept. Ob/Gyn, 2500 N. State St., Jackson, MS 39216
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Rosa-Vazquez, Cesar, 6505 Morningside, El Paso, TX 79904
Shapiro, Alan Isaiah, Oakland Naval Hosp., Box 3708, Oakland, CA 94627
Strickland, Daniel M., 7606 Lynn Ann Lane, San Antonio, TX 78240
Witten, Barry I., 14219 Kinderhook Dr., Chesterfield, MO 63017

New certifications: Subspecialty of Gynecologic Oncology

Date of examination: February 1987

Place: San Francisco, California

Number certified this examination: 21*

Cumulative total of certificates awarded for this subspecialty: 315

**The name of one newly certified diplomate is not included in this listing at his/her request.*

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Macfee, Michael Scott, MacDonald Hosp. for Women, 2105 Adelbert Road, Cleveland, OH 44106
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Welander, Charles Eric, Bowman Gray, Dept. Ob/Gyn, 300 S. Hawthorne Road, Winston-Salem, NC 27103
Wheelock, John B., 131 Watersedge Dr., Ocean Springs, MS 39564

Books received

Adolescent Abortion: Psychological and Legal Issues. Edited by Gary B. Melton. 152 pages. Lincoln, Nebraska, 1986, The University of Nebraska Press. $17.50.

An Introduction to Fetal Physiology. Frederick C. Battaglia and Giacomo Meschia. 257 pages, illustrated. Orlando, 1986, Academic Press, Inc. $45.00.

Anesthesia for Obstetrics. Second Edition. Sol M. Shnider and Gershon Levinson. 566 pages, illustrated. Baltimore, 1987, The Williams & Wilkins Company. $65.95.

Atlas of Fetal Sectional Anatomy. G. Isaacson, M. C. Mintz, and E. S. Crelin. 184 pages, illustrated. New York, 1986, Springer-Verlag, $89.00.

Atlas of Ultrasonographic Artifacts & Variants. Roger C. Sanders. 156 pages, illustrated. Chicago, 1986, Year Book Medical Publishers. No price listed.

A Classified Bibliography of Controlled Trials in Perinatal Medicine 1940-1984. Prepared by the National Perinatal Epidemiology Unit, Oxford, United Kingdom, with the support of the World Health Organization. 414 pages. New York, 1985, Oxford University Press. No price listed.

Brought to Bed. Child-Bearing in America, 1750-1950. Judith Walzer Leavitt. 284 pages. New York, 1986, Oxford University Press. $21.95.

Drug and Chemical Action in Pregnancy. Edited by Sergio Fabro and Anthony R. Scialli. 524 pages, illustrated. New York, 1986, Marcel Dekker, Inc. $89.75.

Drug Therapy in Obstetrics and Gynecology. William F. Rayburn and Frederick P. Zuspan. 513 pages, illustrated. Norwalk, Connecticut, 1986, Appleton-Century-Crofts. No price listed.

Drug Use in Pregnancy: Mother and Child. Ira J. Chasnoff. 163 pages. Lancaster, England, 1986, MTP Press Limited. No price listed.

Extrauterine Pregnancy. Edited by Alvin Langer and Leslie Iffy. 351 pages, illustrated. Littleton, Massachusetts, 1986, PSG Publishing Company, Inc. $35.00.

Handbook for Prescribing Medications During Pregnancy. Second Edition. Richard L. Berkowitz, Donald R. Coustan, and Tara K. Mochizuki. 406 pages. Boston, 1986, Little Brown & Company. No price listed (soft cover).

Health Education for Women. Vivian M. Littlefield. 752 pages, illustrated. East Norwalk, Connecticut, 1986, Appleton-Century-Crofts. $36.95.

Health Risks. Elliott J. Howard with Susan A. Roth. 246 pages. Tucson, 1986, The Body Press. $19.95.

Heart Disease and Pregnancy. Second Edition. James Metcalfe, John H. McAnulty, and Kent Ueland. 432 pages, illustrated. Boston, 1986, Little, Brown & Company. $70.00.

The Human Placenta: Clinical Perspectives. J. Patrick Lavery. 288 pages. Rockville, Maryland, 1987, Aspen Publishers. No price listed.

Introduction to Radiological Physics and Radiation Dosimetry. Frank Herbert Attix. 607 pages, illustrated. New York, 1986, John Wiley & Sons, Inc. $54.95.

Pathology of the Vulva and Vagina. Volume 9, Contemporary Issues in Surgical Pathology. Edited by Edward J. Wilkinson. New York, 1986, Churchill Livingstone, Inc. $55.00.

PMS: A Positive Program to Gain Control. Stephanie DeGraff Bender and Kathleen Kelleher. 245 pages. Tucson, 1986, The Body Press. $7.95 (soft cover).

Pregnancy for Older Women. Phyllis Kernoff Mansfield. 240 pages. New York, 1986, Praeger Publishers. $35.00.

Quantitative Obstetrical Ultrasonography. Russell L. Deter, Ronald B. Harrist, Jason C. Birnholz, and Frank P. Hadlock. 377 pages, illustrated. New York, 1986, John Wiley & Sons. $45.00.

Steroid Hormone Resistance: Mechanisms and Clinical Aspects. Edited by George P. Chrousos, D. Lynn Loriaux, and Mortimer B. Lipsett. 439 pages. New York, 1986, Plenum Press. $69.50.

Transsexualism and Sex Reassignment. Edited by William A. W. Walters and Michael W. Ross. 191 pages, illustrated. New York, 1986, Oxford University Press. $27.95.

When a Baby Dies: A Handbook for Healing and Helping. Rana K. Limbo and Sara Rich Wheeler. 150 pages. La Crosse, Wisconsin, 1986, La Crosse Lutheran Hospital. No price listed (soft cover).

1986 Yearbook of Obstetrics and Gynecology. Roy M. Pitkin and Frank J. Zlatnik. 539 pages, illustrated. Chicago, 1986, Year Book Medical Publishers. No price listed.

ITEMS

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60.00 U.S. per insertion and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146.

Specialty Review in Neonatology/Perinatology, August 24-28, 1987, Chicago, Illinois. Sponsored by Cook County Graduate School of Medicine. For additional information, contact the Registrar's Office. Toll-free number: Illinois 1 (800) 621-4649; outside Illinois 1 (800) 621-4651.

The American Board of Laser Surgery announces its 2nd Certifying Examination on Saturday, October 10, 1987, at the University of California Medical Center, San Francisco, California. Candidates may obtain information and applications from the President of the Board, Stanley Stellar, M.D., St. Barnabas Medical Center, Livingston, NJ 07039, or the Assistant to the President, Mary Cayton, R.N., 3201 So. 16th St., Suite 2006, Milwaukee, WI 53215.

Three Postgraduate Gynecology Courses: Advances in Gynecologic Oncology, September 12-14, 1987; Advances in Gynecology, September 15-17, 1987; Reproductive Endocrinology, September 18-21, 1987; Massachusetts General Hospital, Boston, Massachusetts. For information contact: Harvard Medical School, Department of Continuing Education, 25 Shattuck St., Boston, MA 02115.

International Symposium on First Trimester Genetic Diagnosis and Therapy: Current Status and Future Prospects, September 17-19, 1987, Hilton Hotel and Towers, Chicago, Illinois. Sponsored by the Medical and Reproductive Genetics Section, Eugene Pergament, Director, Department of Obstetrics and Gynecology, Illinois Masonic Department of Obstetrics and Gynecology. For information contact: Janet Dalzell, Illinois Masonic Medical Center, 836 West Wellington St., Chicago, IL 60657. Tel.: (312) 883-7045.

First Congress of the International Society of Gynecological Endocrinology, Crans-Montana, Switzerland, March 6-12, 1988. For information contact the Scientific Secretariat/Organization: A. Volpe, F. Facchinetti, C. Nappi, or F. Petraglia at the Department of Obstetrics and Gynecology, University of Modena, Via del Pozzo, 71-41100 Modena, Italy. Tel.: (39) (59) 360224-360255-379515-379512.

Specialty Review in Obstetrics and Gynecology: Practical Aspects, October 4-10, 1987, Chicago, Illinois. Sponsored by The Cook County Graduate School of Medicine. For additional information contact the Registrar's Office. Toll-free number: Illinois 1(800) 621-4649; outside Illinois 1(800) 621-4651.

Hysteroscopy Workshop: Fundamentals and New Advancements, November 20-21, 1987, Ambassador West Hotel, 1300 North State Parkway, Chicago, IL 60611. For information contact: The Office of Continuing Medical Education, Northwestern University Medical School, 301 E. Chicago Ave., Chicago, IL 60611. Tel.: (312) 908-8533.

Perinatal Medicine 1987: Emergencies in the Mother, Fetus and Neonate, October 8-9, 1987, American Dental Association Auditorium, 211 E. Chicago Ave., Chicago, IL 60611. For information contact: The Office of Continuing Medical Education, Northwestern University Medical School, 301 E. Chicago Ave., Chicago, IL 60611. Tel.: (312) 908-8533.

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PORTANT NOTE—This information is a BRIEF SUMMARY of the complete prescribing information provided with the duct and therefore should not be used as the basis for prescribing the product. This summary was prepared by leting from the complete prescribing information certain text, tables, and references. The physician should be roughly familiar with the complete prescribing information before prescribing the product.

ICATIONS AND USAGE: PREVENTION OF PREGNANCY.

SE-RELATED RISK OF THROMBOEMBOLISM FROM ORAL CONTRACEPTIVES: Two studies have shown a positive ociation between the dose of estrogens in oral contraceptives and the risk of thromboembolism. For this reason, it is dent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive duct prescribed for any given patient should be that product which contains the least amount of estrogen that is mpatible with an acceptable pregnancy rate and patient acceptance. It is recommended that new acceptors of oral ntraceptives be started on preparations containing .05 mg or less of estrogen.

NTRAINDICATIONS: Oral contraceptives should not be used in women with any of the following conditions: 1. rombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic sorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or spected estrogen-dependent neoplasia. 6. Undiagnosed, abnormal genital bleeding. 7. Oral contraceptive tablets may use fetal harm when administered to a pregnant woman. Oral contraceptive tablets are contraindicated in women who pregnant. If the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard the fetus (see WARNINGS, No. 5). 8. Benign or malignant liver tumor which developed during the use of oral ntraceptives or other estrogen-containing products.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. An increased risk of thromboembolic and rombotic disease associated with the use of oral contraceptives is well established. Four principal studies in Great itain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism d stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times ore likely than nonusers to develop these diseases without evident cause. Overall excess mortality due to pulmonary nbolism or stroke is on the order of 1.0 to 3.5 deaths annually per 100,000 users and increases with age. **EREBROVASCULAR DISORDERS:** In a collaborative American study of cerebrovascular disorders in women with and thout predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than in nusers and the risk of thrombotic stroke was 4.0 to 9.5 times greater in users than in nonusers. A prospective study nducted in Great Britain estimated that former users have a risk for all cerebrovascular disease 2.6 times greater than at of nonusers. This risk remained elevated for at least six years after last oral contraceptive use. A prospective study onducted in the United States found that past use of oral contraceptives was associated with increased risk of ubarachnoid hemorrhage, the relative risk being 5.3. There was also some evidence from this study that the degree of risk ay be related to duration of oral contraceptive use. **MYOCARDIAL INFARCTION:** An increased risk of myocardial nfarction associated with the use of oral contraceptives has been reported confirming a previously suspected ssociation. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, iabetes, history of preeclamptic toxemia), the higher the risk of developing myocardial infarction, regardless of whether he patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk actor. The annual excess case rate (increased risk) of myocardial infarction (fatal and nonfatal) in oral contraceptive sers was estimated to be approximately 7 cases per 100,000 women users in the 30-39 age group and 67 cases per 00,000 women users in the 40-44 age group. In terms of relative risk, it has been estimated that oral contraceptive users vho do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as kely to have a fatal myocardial infarction as nonusers who do not smoke. Oral contraceptive users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke, but about a 10- to 12-fold ncreased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. n determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and bsolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a esser extent. A study suggests that some increased risk of myocardial infarction in oral contraceptive users persists ollowing discontinuation of oral contraceptives and that the degree of the residual risk is related to the duration of the ast use. **Risk of Dose:** In an analysis of data derived from several national adverse reaction reporting systems, British nvestigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives. Preparations containing 100 mcg or more of estrogen were associated with a higher isk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States. Careful epidemiological studies to determine the degree of thromboembolic risk associated with progestogen-only oral contraceptives have not been performed. Cases of thromboembolic disease have been reported in women using these products, and they should not be presumed to be free of excess risk. The risk of thromboembolic and thrombotic disorders, in both users and nonusers of oral contraceptives, increases with age. Oral contraceptives are, however, an independent risk factor for these events. **ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES:** A large prospective study carried out in the United Kingdom estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of oral contraceptives according to age, smoking habits, and duration of use. The overall excess death rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking, and duration of use, nor to compare the effects of continuous versus intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for five or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with ontinuous use for five or more years are available, it is not possible to assess the magnitude of the relative risk for this ounger age group. This study reports that the increased risk of circulatory disease mortality may persist after the pill is iscontinued. Another study published at the same time confirms a previously reported increase of mortality in pill users om cardiovascular disease. The study concluded that the mortality associated with all methods of birth control is low d below that associated with childbirth, with the exception of oral contraceptives in women over 40 who smoke. (The tes given for pill only/smokers for each age group are for smokers as a class. For "heavy" smokers [more than 15 garettes a day], the rates given would be about double; for "light" smokers [less than 15 cigarettes a day], about 50 rcent.) The mortality associated with oral contraceptive use in nonsmokers over 40 is higher than with any other ethod of contraception in that age group. The lowest mortality is associated with the condom or diaphragm backed up y early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with ge after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolmia, obesity, diabetes, or history of preeclamptic toxemia and especially by cigarette smoking. The risk of myocardial nfarction in oral contraceptive users is substantially increased in women age 40 and over, especially those with other risk actors. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic isorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal rombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued mmediately. A four- to six-fold increased risk of postsurgery thromboembolic complications has been reported in oral ontraceptive users. If feasible, oral contraceptives should be discontinued at least four weeks before surgery of a type ssociated with an increased risk of thromboembolism or prolonged immobilization. 2. OCULAR LESIONS. There have een reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral ntraceptives. Discontinue oral contraceptive medication if there is unexplained, sudden or gradual, partial or complete s of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions and institute appropriate diagnostic d therapeutic measures. 3. CARCINOMA. Long-term continuous administration of either natural or synthetic estrogen certain animal species increases the frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic rogestogens, none currently contained in oral contraceptives, have been noted to increase the incidence of mammary dules, benign and malignant, in dogs. In humans, three case control studies have reported an increased risk of ndometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One ublication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on oral contraceptives. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time oral contraceptives were first given, polycystic ovaries), nearly all occurred in women who had used a sequential oral contraceptive. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only oral contraceptives. Several studies have found no increases in breast cancer in women taking oral contraceptives or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with oral contraceptives, found an excess risk in the subgroups of oral contraceptive users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of oral contraceptives has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. 4. HEPATIC TUMORS. Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study showed that oral contraceptive formulations with high hormonal potency were associated with a higher risk than lower potency formulations and use of oral contraceptives with high hormonal potency and age over 30 years may further increase the woman's risk of hepatocellular adenoma. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of oral contraceptives. Two studies relate risk with duration of use of the contraceptive, the risk being much greater after four or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. Cases of hepatocellular carcinoma, although rare, have been reported in women taking oral contraceptives. Retrospective studies conducted in the United States and Britain report an increased risk of hepatocellular carcinoma in oral contraceptive users, particularly with increased duration of use. 5. USE IN OR IMMEDIATELY PRECEDING PREGNANCY, BIRTH DEFECTS IN OFFSPRING, AND MALIGNANCY IN FEMALE OFFSPRING. The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be on the order of 1 to 4 in 1000 exposures. Although there is no evidence at the present time that oral contraceptives further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including oral contraceptives, in pregnancy. One case control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing oral contraceptives. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the last menstrual period if the progestogen-only oral contraceptives are used), and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy. 6. GALLBLADDER DISEASE. Studies report an increased risk of surgically confirmed gallbladder disease in users of oral contraceptives and estrogens. In one study, an increased risk appeared after two years of use and doubled after four or five years of use. In one of the other studies, an increased risk was apparent between six and twelve months of use. 7. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. An increase in triglycerides and total phospholipids has been observed in patients receiving oral contraceptives. The clinical significance of this finding remains to be defined. 8. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in patients receiving oral contraceptives. In some women hypertension may occur within a few months of beginning oral contraceptive use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two and a half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in oral contraceptive users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. Hypertension that develops as a result of taking oral contraceptives usually returns to normal after discontinuing the drug. 9. HEADACHE. The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of oral contraceptives and evaluation of the cause. 10. BLEEDING IRREGULARITIES. Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Postuse anovulation, possibly prolonged, may also occur in women without previous irregularities. 11. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. 12. BREAST FEEDING. Oral contraceptives given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of oral contraceptives should be deferred until the infant has been weaned. **PRECAUTIONS: General:** 1. A complete medical and family history should be taken prior to the initiation of oral contraceptives. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than one year without another physical examination being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by oral contraceptive therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping oral contraceptives, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. **INFORMATION FOR THE PATIENT: (See Patient Package Insert). DRUG INTERACTIONS:** Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin, griseofulvin, and tetracycline. **DRUG/LABORATORY TEST INTERACTIONS:** The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. **CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY, PREGNANCY, NURSING MOTHERS: See CONTRAINDICATIONS and WARNINGS. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS): Thrombophlebitis. Pulmonary embolism. Coronary thrombosis. Cerebral thrombosis. Cerebral hemorrhage. Hypertension. Gallbladder disease. Liver tumors. Congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis. Neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10% or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Dysmenorrhea. Amenorrhea during and after treatment. Temporary infertility after discontinuance of treatment. Edema. Chloasma or melasma which may persist. Breast changes: tenderness, enlargement, and secretion. Change in weight (increase or decrease). Change in cervical erosion and cervical secretion. Possible diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Increase in size of uterine leiomyomata. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome. Cataracts. Changes in libido. Chorea. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Revised 2/87

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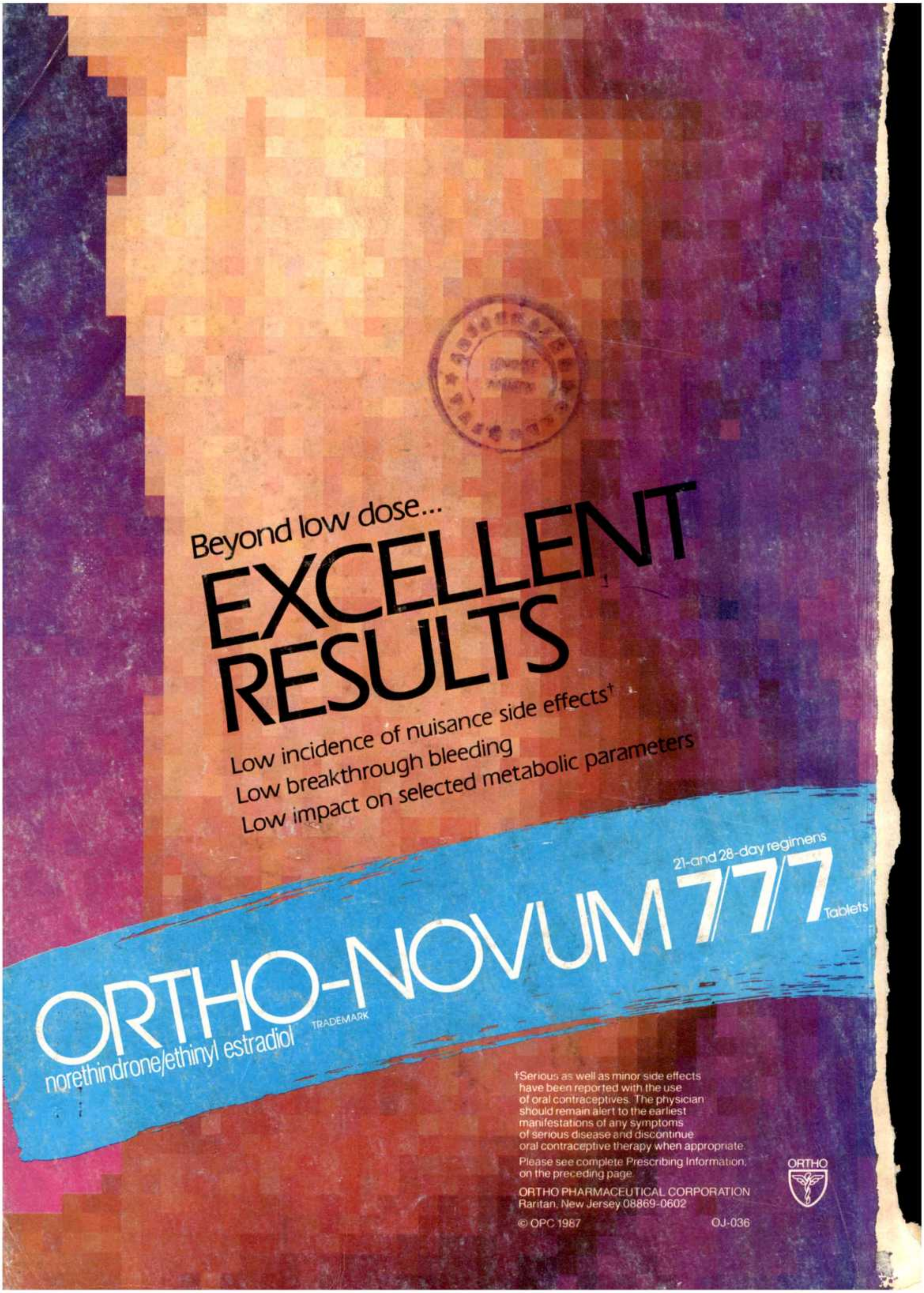
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†Serious as well as minor side effects have been reported with the use of oral contraceptives. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate.

Please see complete Prescribing Information, on the preceding page.

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